The effect of gamma-radiation on hydrocortisone in solutions and topical preparations

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THE EFFECT OF GAMMA-RADIATION ON HYDROCORTISONE IN SOLUTIONS AND TOPICAL PREPARATIONS

Submitted by

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This research has been carried out in the School of Pharmacy and Pharmacology of the University of Bath under the supervision of G. Fletcher, M.Sc., F.P.S. and D.J.G. Davies, M.Sc., Ph.D., F.P.S.

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TO MY PARENTS AND MY WIFE

SUMMARY

The introduction begins with the use of gamma-radiation as a sterilisation method for pharmaceutical preparations. The radiation chemistry of water and the reactivities of the three major radiolytic products of water, namely, the hydroxyl radical, the hydrogen atom and the hydrated electron with chemicals are reviewed. The radiation chemistry of organic solvents is discussed with particular reference to the effect of the resulting radiolytic products of these solutions on solutes such as corticosteroids when exposed to ionising-radiation. This is followed by a discussion on the reactivities of surfactants with the radiolytic species of water and the effect of micellar systems on the thermal and radiolytic stability of solubilisates.

The experimental part is initially concerned with a confirmation of characteristics of Cobalt-60 and Caesium-137 radiation sources which is followed by the development of a specific HPLC assay method for each of the three corticosteroids, Hydrocortisone, Hydrocortisone Acetate and Hydrocortisone Phosphate. It also deals with determining the order of the initial reactions of the three corticosteroids with gamma-radiation. The second part is concerned with the sensitivities of the three corticosteroids to gamma-radiation in aqueous and organic solvents, followed by investigating the effect of a number of free radical scavengers such as oxygen, methanol, 2-propanol

and iodine on the stability of the corticosteroids to radiation. In the third part, the influence of the cationic, anionic and non-ionic surfactants, CTAB, NaLS and Cetomacrogol 1000 respectively on Hydrocortisone and Hydrocortisone Phosphate degradation due to radiation in aqueous system has been studied. This study has been followed by investigating the sensitivity of hydrocortisone in formulated cream and ointment where the influence of the individual ingredients on the drug sensitivity has been demonstrated. The final part is concerned with the separation of the degradation products of the three corticosteroids by HPLC and TLC techniques after exposure to gamma-radiation.

In the concluding section, the experimental results are discussed and the feasibility of using gamma-radiation for sterilising the formulated hydrocortisone cream and ointment is evaluated.

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THE ORIGIN AND THE SCOPE OF THE WORK

THE ORIGIN AND SCOPE OF THE WORK

Some pharmaceutical preparations such as parenteral injections, perfusion fluids and electrolytes, eye drops and some topical preparations are required to be sterile. The standard recommended methods of sterilisation in the British Pharmacopoeia[1] are heat sterilisation, filtration, exposure to gases and exposure to ionising radiation.

Ionising radiation offers several advantages in principle over the other methods. For example, micro-organisms can be inactivated efficiently; materials can be treated at room temperatures or below; appreciable penetration can be achieved so that preparations can be treated inside sealed containers made of such materials as plastic, glass or metals, and the process is suitable for continuous operation. Recent developments towards tighter control on micro-biological standards for all pharmaceuticals have stimulated further interest in the use of ionising radiation, as well as determining its usefulness as an alternative sterilisation method to ethylene oxide gas. For example, the U.S. Food and Drug Administration (FDA) has proposed strict limits on the allowable residual quantities of ethylene oxide and its major reaction products in the drug because of its possible carcinogenic properties[2]. The FDA therefore published a proposal regulating the irradiation of foods for human consumption[3] which permits irradiation of any food at a dose not $\rangle$ 1000 Gray (100K. rad). It also permits the irradiation of foods at a dose of $\leqslant$ 50 K. Gray if the foods comprise only a minor portion (not $\rangle$ 0.01%) of the daily diet.

Information determining the degree of degradation and the identification of the degradation products unique to irradiation should be sufficient to examine the feasibility of ionising radiation as an alternative method for sterilising pharmaceutical materials and products. Several studies have been carried out[2,4,5] to determine the sterilising dose requirements and the British Pharmacopoeia[1] recommends 25 K.Gray (2.5 M rad) as the standard dose for sterilisation.

Investigations of the effect of ionising radiation on drugs in different solvents and topically applied bases have been carried out by many workers[5,6,7,8]. These studies have shown that drugs are generally more stable in the dry state than in organic solvents or aqueous solutions. Drugs in aqueous solutions have particularly been found to be highly sensitive to ionising radiation even at a radiation dose much lower than the sterilisation dose[9,10,11]. This high sensitivity is due to the reactive radiolytic products of water which are capable of inducing chemical changes in the drug. The presence of surfactants in the aqueous solutions of a drug makes the system more complex, consisting of water, surfactant monomers and surfactant micelles. It is likely therefore that the radiation sensitivity of a drug will be different in that system from that of simple aqueous solution as shown by a number of workers[12,13].

The purpose of the present work was to investigate the feasibility of sterilising hydrocortisone, in topical preparations such as ointments and creams, by ionising radiation. Because Hydrocortisone, is a slightly water soluble alcohol, it was felt necessary in this study to include two

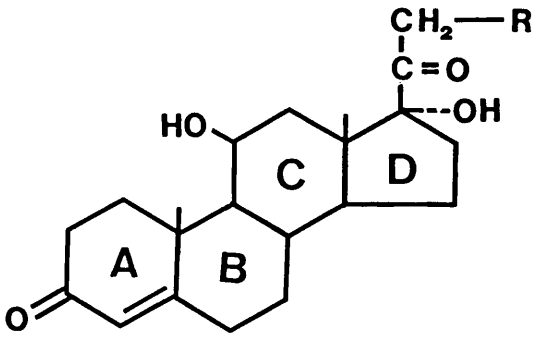
of its esters, the acetate which is insoluble in water and phosphate which is water soluble to obtain a more comprehensive study of the drug and its esters in solvents of different polarities.

It is particularly desirable for topical preparations containing corticosteroids to be presented to the patient in a sterile form as these preparations may come in contact with wound areas or open skin surfaces where the introduction of infection and its growth would be greatly enhanced by the application of non sterile formulations, particularly because of the immuno-suppressive nature of corticosteroids.

Hydrocortisone (I) is topically used in the treatment of various skin disorders and presented in the British Pharmacopoeia in topical preparations such as ointments, creams and lotions containing 0.25-2.5% of the drug. In the cream, hydrocortisone is uniformly dispersed in a water miscible cetomacrogol cream base and it might be expected that the water content in the formula may cause some degree of degradation of the drug through the produced radiolytic species of water. On the other hand, in the ointment preparation, hydrocortisone may be directly dispersed into white soft paraffin as in the British Pharmacopoeia[1] or dissolved in propylene glycol and then uniformly dispersed in white soft paraffin as in the Nordic Pharmacopoeia. The presence of propylene glycol however may influence the sensitivity of the drug to ionising radiation, as shown by Hayes[6] in her study on the sensitivity of Beclomethasone dipropionate to gamma-radiation.

Hydrocortisone acetate (II) has the same uses as hydrocortisone and is officially available in the form of ointments, creams and lotions containing 0.25-2.5%. Also, it is given by intra-articular injection into joints affected by rheumatoid arthritis.

Hydrocortisone phosphate (III) which is water soluble and administered by injection in allergic emergencies, is usually sterilised by filtration as it is a safe method for sterlising thermolabile drugs. Such injections of hydrocortisone acetate and hydrocortisone phosphate require to be sterile, therefore a knowledge of the effect of gamma-radiation as a method of sterilisation would be of value.



(I) R = OH Hydrocortisone

(II) R = $OCOCH_3$ Hydrocortisone 21-acetate

(III) R = Na_2PO_4 Hydrocortisone 21-disodium phosphate

1. INTRODUCTION

INTRODUCTION

Nature and Source of Gamma-Radiation

Gamma rays are naturally occurring electromagnetic radiations with a wavelength of 10-100Å. One of the earliest sources of such radiation was radium, but being rare and expensive, it is more usual now to use artificial radioactive sources, which can be made thousands of times more powerful than the biggest radium source. One of the most widely used is Cobalt-60 which emits two gamma rays with energies of 1.17 and 1.33 Mev. and has a half-life of 5.3 years. Amongst other artificial gamma ray sources is Caesium-137 which emits γ-rays with an energy of 0.66 Mev. and a half-life of 30 years[14]. As an alternative to radioactive isotopes, electron generators are also used in radiation chemistry. They have higher energies than those emitted by Cobalt-60 and Caesium-137 (3-25 Mev.). Although more maintenance is required for electron generators, some workers prefer them for their reduced outlay costs and safer use because the ionising radiation can be stopped and terminated at any time[15].

Chemical Effect of Gamma-Radiation

The chemical effect of electromagnetic radiations is due to the fast electrons produced. These radiations lose their energy in photons to the surrounding matter in three ways:

1. Photoelectric absorption: When γ-rays have a low energy (below o.1 Mev.), all the energy of the photon is absorbed by an atom which ejects an electron, usually from one of the inner shells. These electrons react with the surrounding

atoms causing excitation and ionisation.

2. Compton Scattering: When γ-rays have an energy range of 0.1-2.0 M ev., they interact with an atom and impart only some of their energy. The electron is ejected and the photon with less energy and different wavelength is free to react with further atoms by either the photoelectric effect or the Compton Scattering method. This is the most common mechanism of loss of energy by electromagnetic radiations in organic systems.

3. Pair production: At energies above 1.02 M ev., photons can interact with the nucleus and an electron-positron pair is formed which causes ionisation and excitation.

Generally, the ionising radiation may react with chemicals in solution in two ways, "direct action" or "indirect action". The former occurs when molecules, undergoing chemical changes, are ionised or excited directly by an electron. On the other hand, "indirect action" assumes that the chemical effect on molecules is brought about by the highly reactive products resulting from the deposition of energy on solvent molecules. This action can readily occur in dilute aqueous solution, where the number of water molecules is large while the number of solute molecules is relatively small. The radiolytic products of the solvent then react with solute molecules. Therefore "direct action" may occur in the irradiation of pure substances in the solid state, whereas in aqueous solution both direct and indirect actions can occur, although the probability of "indirect action" is generally much greater.

The overall effect of the passage of ionising radiation through matter is therefore the formation of ions and electronically excited molecules. The excited molecules may dissipate their energy by transference to another molecule, with the emission of light as fluorescence or by dissociation with the formation of free radicals. The free radicals and ions formed by this "primary process" are then capable of taking part in a number of secondary chemical processes[16]:

1. Dissociation:

$$R^{\bullet} \longrightarrow R_a^{\bullet} + R_bH$$

$$CH_3O^{\bullet} \longrightarrow H^{\bullet} + CH_2O$$

2. Abstraction:

$$R^{\bullet} + MH \longrightarrow M^{\bullet} + RH$$

$$H^{\bullet} + CH_3OH \longrightarrow {}^{\bullet}CH_2OH + H_2$$

3. Recombination:

$$R_1^{\bullet} + R_2^{\bullet} \longrightarrow R_1\,R_2$$

$${}^{\bullet}CH_2OH + {}^{\bullet}H \longrightarrow CH_3OH$$

4. Disproportionation:

$$R_1^{\bullet} + R_2^{\bullet} \longrightarrow R_1H + R_2H$$

$$CH_3\dot{O} + CH_3\dot{C}HOH \longrightarrow CH_3OH + CH_3CHO$$

5. Dimerisation:

$$R^{\bullet} + R^{\bullet} \longrightarrow (R)_2$$

$$\dot{C}H_2OH + \dot{C}H_2OH \longrightarrow (CH_2OH)_2$$

Units of Measurement

The unit of absorbed dose of radiation, generally used until recently, was the "rad", which is equal to 100 ergs of energy absorbed by one gramme of the irradiated material. At the request of the International Commission on Radiation Units of measurements, the term "Gray" (symbol Gy) has been accepted as a special name for the Joule per Kilogram to replace the rad as the unit dose of absorbed radiation and became official in 1985. One gray is equivalent to 100 rad, and therefore the British Pharmaceopoeia 1981 quotes the recommended sterilising dose for ionising radiation as 25 K.Gy (2.5 M.rad).

To compare the effect of radiation on different materials, the term G^{+}_{-} value is used. This value indicates the number of molecules of material formed (G^{+}) or destroyed (G^{-}) by 100 ev. of radiation energy deposited in the substance. The radiation energy of one rad equals 6.24×10^{13} ev. per gramme of substance[17]. For most radiation induced chemical reactions the G^{+}_{-} values range from 1-5[18].

Primary Products of Water Radiolysis

The decomposition of water by ionising radiation has been investigated by several workers[14,19,20,21,22] and on the basis of their results, Allen[14] has proposed that the irradiation of water, in the first instance, gives energy to the electronic system of the water molecule and then produces ionisation or an excited state:

Ionisation of water: $H_2O \rightsquigarrow H_2O^{+} + \bar{e}$

Excitation of water: $H_2O \rightsquigarrow H_2O^{*}$

Subsequently the ionised and the excited water molecules undergo further reactions to produce hydrogen atoms, hydroxyl radicals and hydroxide ions.

$$H_2O^{+} + H_2O \longrightarrow H_3O^{+} + \dot{O}H$$

$$\bar{e} + H_2O \longrightarrow H^{\cdot} + \bar{O}H$$

$$H_2O^{*} \longrightarrow H^{\cdot} + \dot{O}H$$

Some of these species can interact with each other to give molecular products.

$$H^{\cdot} + H^{\cdot} \longrightarrow H_2$$

$$\dot{O}H + \dot{O}H \longrightarrow H_2O_2$$

$$H^{\cdot} + \dot{O}H \longrightarrow H_2O$$

Czapski[23], from his experimental work, has demonstrated that the reducing radical produced in water radiolysis has a unit negative charge and can be considered to be a solvated electron, designated $H_2\bar{O}$, and Collinson[24] and Barr[25] have concluded that the hydrogen atom can exist in three forms H_a, H_b and H_c and that the relationship between them is

$$H_a + H^{+} \longrightarrow H_b$$

$$H_b + H^{+} \longrightarrow H_c$$

These forms can only be

$$H_a = \bar{e}_{aq.}\,;\ H_b = \dot{H} \text{ and } H_c = H_2^{+}$$

Therefore the overall primary chemical result from the irradiation of water is

$$H_2O \overset{\sim\sim}{\longrightarrow} H^{\cdot},\ \bar{e}\ aq.,\ \dot{O}H,\ H_3O^{+},\ H_2 \text{ and } H_2O_2$$

Effect of Oxygen Content on Irradiation of Aqueous Solutions

The presence of O_2 drastically changes the radiation chemistry of dilute aqueous solutions and alters that of concentrated solutions as well[26,27].

$$\bar{e}aq. + O_2 \longrightarrow \dot{O}_2^- \qquad 1.8 \times 10^{10} M^{-1} Sec^{-1} \quad ....A$$

$$\dot{H} + O_2 \longrightarrow H\dot{O}_2 \qquad 2.0 \times 10^{10} M^{-1} Sec^{-1} \quad ....B$$

The high rate constants of reactions A and B indicate that oxygen, even in air-saturated solutions, reacts principally with the reducing radicals, $\bar{e}aq.$ and $\dot{H}$ giving superoxide ion and hydroperoxy free radical respectively. These products can undergo further reactions as follows:

$$\dot{O}_2^- + \dot{O}H \longrightarrow \bar{O}H + O_2$$

$$\dot{O}_2^- + H\dot{O}_2 \longrightarrow H\dot{O}_2^- + O_2$$

$$H\dot{O}_2 + H\dot{O}_2 \longrightarrow H_2O_2 + O_2$$

$$H\dot{O}_2 + \dot{O}H \longrightarrow H_2O_3$$

$$H\dot{O}_2 + \bar{e}_{aq} \longrightarrow H\dot{O}_2^-$$

$$H\dot{O}_2 \rightleftharpoons H^+ + \dot{O}_2^-$$

Thus, the hydroperoxy radical $H\dot{O}_2$, is a very effective oxidising agent, and in acid solution, it will further react with the hydrogen ions to give hydrogen peroxide:

$$H\dot{O}_2^- + H^+ \longrightarrow H_2O_2$$

In the absence of oxygen, an organic radical $\dot{R}$, can only undergo the following reactions:-

1. Dimerisation.
2. Disproportionation.
3. Reaction with another radical.

Effect of pH

Variation of the pH may greatly affect the products of water radiolysis. This effect can be summarised as:

a) In acid medium (pH 2.1 - 4.3) the hydrated electron may be effectively converted into the hydrogen atom:

$$\bar{e}_{aq} + H_3O^+ \longrightarrow \dot{H} + H_2O$$

b) In alkaline medium, the hydroxyl radical is converted into the ion radical $\dot{O}^-$ [25]:

$$\dot{O}H + \bar{O}H \longrightarrow \dot{O}^- + H_2O$$

and the hydrogen atom is converted to the hydrated electron at pH 11 - 13 [28]

$$\dot{H} + \bar{O}H \longrightarrow \bar{e}_{aq.} + H_2O$$

Chemical Reactivities of the Free Radicals Induced by Water Radiolysis

A - The hydrogen atom is capable of undergoing the following chemical reactions with other molecules:

1. Abstraction of hydrogen:

$$\dot{H} + CH_3COOH \longrightarrow H_2 + \dot{C}H_2COOH$$

2. Deamination:

$$\dot{H} + H_2N\,CH_2COOH \longrightarrow NH_3 + \dot{C}H_2COOH$$

3. Addition to unsaturated compounds:

$$\dot{H} + -CH = CH- \longrightarrow -\dot{C}H-CH_2-$$

4. Reduction:

$$\dot{H} + Cu^{++} \longrightarrow Cu^{+} + H^{+}$$

B The hydrated electron, however, undergoes the following types of reactions:

1. Reduction:

$$Cu^{++} + \bar{e}_{aq.} \longrightarrow Cu^{+}$$

2. Electron transfer to an electronegative group such as carbonyl group:

$$\bar{e}_{aq} + CH_3\text{-}CO\text{-}CH_3 \longrightarrow CH_3\text{-}\dot{C}O\text{-}CH_3 \xrightarrow{H_2O} CH_3\text{-}\underset{\displaystyle OH}{\overset{\displaystyle \cdot}{C}}\text{-}CH_3 + \dot{O}H$$

$$2CH_3\text{-}\underset{\displaystyle OH}{\overset{\displaystyle \cdot}{C}}\text{-}CH_3 \longrightarrow CH_3\text{-}\underset{\displaystyle OH}{CH}\text{-}CH_3 + CH_3\text{-}CO\text{-}CH_3$$

C <u>The hydroxyl radical</u> is considered as a strong oxidising species and can undergo the following reactions:

1. Abstraction of a hydrogen atom:

$$\dot{O}H + CH_3\ CH_2OH \longrightarrow CH_3\ \dot{C}HOH + H_2O$$

2. Addition to an unsaturated compound:

$$\dot{O}H + -CH = CH- \longrightarrow -\underset{\displaystyle OH}{CH}\text{-}\dot{C}H-$$

3. Oxidation:

$$\dot{O}H + Fe^{++} \longrightarrow Fe^{+++} + \bar{O}H$$

<u>Radiation Chemistry of Alcohols</u>

The products formed in the irradiation of methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl and tertiary butyl alcohols have been reported as[29]:-

1 <u>Reduced products</u>: such as hydrogen and saturated hydrocarbons. The mechanism of formation of hydrogen has been gained by analysing the gas from partially deuterated alcohols and it has been found that the main primary fission occurs at the α-carbon group[30,31].

$$CH_3OH \rightsquigarrow \dot{C}H_2OH + H^{\cdot}$$

$$H^{\cdot} + CH_3OH \longrightarrow \dot{C}H_2OH + H_2$$

$$H^{\cdot} + CH_3CH_2OH \longrightarrow CH_3\dot{C}HOH + H_2$$

The hydrocarbons are formed through the breakdown of C-C bonds.

2 Oxidised products:

Bolt and Carroll[18,32] have reported that, as a result of ionising radiation, aldehydes and glycols are produced from primary alcohols, whereas aldehydes, ketones and glycols are produced from secondary alcohols. Ketones and glycols (in minor amounts) being produced from tertiary alcohols. The glycols formed are only of α-type due to the predominance of breakdown at the α-carbon atom:

$$2\ \dot{C}H_2OH \longrightarrow (CH_2OH)_2$$

Further oxidation of the organic radicals however results in formation of carbonyl compounds[18]

$$\dot{C}H_2OH \longrightarrow CH_2O + H^{\bullet}$$

3 Minor products: Carbon monoxide and water.

Production of H_2O shows the probability of scission of carbon-oxygen bond[33].

The Radicals produced during γ-radiolysis of several alcohols have been trapped by reaction with 2-nitroso-2-methyl propane (t-nitrosobutane) to give nitroxides which had been detected by e.s.r.[34].

$$R^{\bullet} + \text{t-BuNO} \longrightarrow \text{t-BuN(}\dot{O}\text{)-R}$$

All alcohols gave alkoxy radicals and hydroxyalkyl-radicals derived by the loss of a hydrogen atom from the carbon adjacent to the OH group (α-c). The alkoxy radical may be the precursor for the formation of hydroxyalkyl radical:

$$CH_3\overset{+}{O}H + CH_3OH \longrightarrow CH_3\dot{O} + CH_3\overset{+}{O}H_2$$

$$CH_3\dot{O} + CH_3OH \longrightarrow \dot{C}H_2OH + CH_3OH$$

Generally, the alkyl chain length as well as the degree of branching largely affects the reactivity of alcohols[6]. The yield of hydrocarbons, aldehydes and glycols from primary alcohols decreases as the chain length of the alkyl chain increases, while the hydrogen yield remains constant. This would indicate that bonds are broken elsewhere in the molecule than the α-c, and the OH group has less effect as the molecule behaves more as a hydrocarbon. As the branching in the alkyl chain increases, the activity of the alcohol increases, which may be explained as an inductive effect which tends to increase the electron density at the α-carbon position and makes abstraction easier.

The effect of oxygen on radiolysis of alcohols has been investigated[35,36] and the yields of the irradiation of deaerated Methanol were mainly found to be H_2, ethylene glycol and formaldehyde with smaller amounts of CO and CH_4. While in oxygen saturated Methanol, the products formed were formaldehyde and hydrogen peroxide with a smaller amount of H_2:-

In the absence of O_2:

$$CH_3\overset{+}{O}H \longrightarrow \dot{C}H_2OH + H^+$$

$$CH_3\bar{O}H \longrightarrow CH_3\bar{O} + H^\cdot$$

$$CH_3\bar{O}H + H^+ \longrightarrow CH_3OH + H^\cdot$$

$$CH_3\bar{O} + H^+ \longrightarrow CH_3OH$$

$$2\dot{C}H_2OH \longrightarrow (CH_2OH)_2$$

$$\dot{C}H_2OH \longrightarrow CH_2O + H^\cdot$$

$$2H^\cdot \longrightarrow H_2$$

$$H^\cdot + CH_3OH \longrightarrow \dot{C}H_2OH + H_2$$

In the present of O_2:

$$CH_3\dot{O}H + O_2 \longrightarrow \dot{O}_2^- + CH_3OH \quad \text{superoxide anion}$$

$$\dot{H} + O_2 \longrightarrow H\dot{O}_2 \quad \text{hydroperoxy radical}$$

$$\dot{C}H_2OH + H\dot{O}_2 \longrightarrow CH_2O + H_2O_2 \qquad \text{.....(1.1)}$$

$$CH_3OH + \dot{H} \longrightarrow \dot{C}H_2OH + H_2$$

$$\dot{C}H_2OH + O_2 \longrightarrow CH_2OH\,(O\text{-}O\cdot) \xrightarrow{\bar{O}_2} CH_2OH\,(O\text{-}O^-) \qquad \text{.....(1.2)}$$

$$CH_2OH\,(O\text{-}O^-) \xrightarrow{H^+} H\,COOH + H_2O$$

As the O_2 concentration increases, reaction (1.1) occurs in preference to reaction (1.2).

Stockhausen *et al*[37] have suggested that most organic radicals readily add O_2 and the peroxy radicals formed undergo ionisation and dimerisation.

$$CH_3OH + \dot{O}H \longrightarrow \dot{C}H_2OH + H_2O$$

$$\dot{C}H_2OH + O_2 \longrightarrow CH_2(OH)\,\dot{O}_2$$

$$CH_2(OH)\,\dot{O}_2 \rightleftharpoons CH_2\,(\bar{O})\,\dot{O}_2 + H^+$$

$$2CH_2(OH)\,\dot{O}_2 \longrightarrow CH_2(OH)\,O_4H + HCHO$$

Radiation Chemistry of Propylene Glycol

The radiolysis products of propylene glycol in air and in nitrogen have been reported by Schwenker[38] indicating that acetone and water are the major radiolytic products in air, while in nitrogen the yields of these two products are reduced. Therefore, propylene glycol is more stable when irradiated in nitrogen than in air. Due to the presence of two hydroxyl

groups in the glycol, it can be expected that a greater number of radicals are produced, as shown in table 1.1.

Table 1.1 THE POSSIBLE DISSOCIATION PRODUCTS OF PROPYLENE GLYCOL EXPOSED TO IONISING RADIATION

POSSIBLE DISSOCIATION PRODUCTS OF PROPYLENE GLYCOL		
LOSS OF $H^{\cdot}$	LOSS OF $\dot{O}H$	ORGANIC RADICALS
$CH_3\dot{C}(OH)CH_2OH$	$CH_3CH(OH)\dot{C}H_2$	$\dot{C}H_3 + \dot{C}H(OH)CH_2OH$
$CH_3CH(OH)\dot{C}HOH$	$CH_3\dot{C}HCH_2OH$	$CH_3\dot{C}HOH + \dot{C}H_2OH$
$\dot{C}H_2CH(OH)CH_2OH$		
$CH_3CH(\dot{O})CH_2OH$		
$CH_3CH(OH)CH_2\dot{O}$		

In aqueous solution, propylene glycol is probably attacked by the radiolytic products of water, as in the case of aliphatic alcohols, to produce the following possible products:-

$$CH_3CH(OH)CH_2OH \begin{cases} \longrightarrow CH_3\dot{C}(OH)CH_2OH \longrightarrow CH_3COCH_2 \longrightarrow CH_3COCH_3 \\ \longrightarrow CH_3CH(OH)\dot{C}HOH \longrightarrow CH_3\dot{C}HCHO \longrightarrow CH_3CH_2CHO \end{cases}$$

Ahmed et al[39], who investigated the γ- radiolysis of ethylene glycol in aqueous solution, have reported that only hydroxyacetaldehyde and hydrogen peroxide could be detected as shown in the following reactions:-

$$\dot{H} + O_2 \longrightarrow \dot{H}O_2$$

$$\bar{e}_{aq} + O_2 \longrightarrow \bar{O}_2$$

$$\bar{e}_{aq} + H_3^+O \longrightarrow \dot{H} + H_2O$$

$$2\,HO_2^{\cdot} \longrightarrow H_2O_2 + O_2$$

$$OH^{\cdot} + HOCH_2CH_2OH \longrightarrow CH^{\cdot}(OH)CH_2OH + H_2O$$

$$O_2 + CH^{\cdot}(OH)CH_2OH \longrightarrow O\text{-}O\text{-}CH^{\cdot}(OH)CH_2OH$$

$$O\text{-}O\text{-}CH^{\cdot}(OH)CH_2OH \longrightarrow HOCH_2CHO + HO_2^{\cdot}$$

$$O\text{-}O\text{-}CH^{\cdot}(OH)CH_2OH + HO_2^{\cdot} \longrightarrow HOCH_2CHO + H_2O_2 + O_2$$

Radiation Chemistry of Alcohols in Aqueous Solution

The radiolysis of aqueous solutions of alcohols results in the production of hydrogen, α-glycols and carbonyl compounds[36,40]. The glycols are produced through the attack of radiolytic species of water.

$$H_2O \rightsquigarrow H^{\cdot} + OH^{\cdot}$$

$$H^{\cdot} + CH_3OH \longrightarrow CH_2^{\cdot}OH + H_2$$

$$OH^{\cdot} + CH_3OH \longrightarrow CH_2^{\cdot}OH + H_2O$$

$$CH_2^{\cdot}OH \longrightarrow CH_2O + H^{\cdot}$$

$$2\,CH_2^{\cdot}OH \longrightarrow (CH_2OH)_2$$

From studies done on the radiolysis of aqueous solutions containing 2-propanol and acetone, Allan et al[41] concluded that another reducing species, the solvated electron, could be produced. This conclusion was supported by Hayon[42] who stated that oxygen was able to quench the excited water molecules and convert them to solvated electrons:-

$$O_2 + H_2O^{*} \longrightarrow \bar{e}_{aq.} + OH^{\cdot}$$

The rate constants for the interaction of radiolytic products of water; $\bar{e}aq.$, $H^{\cdot}$, $OH^{\cdot}$, are shown in table 1.2 [27,43,44]

The effect of oxygen on the radiolysis of aqueous solutions of ethanol has been investigated by Seddon et al[45] and the competition of oxygen and ethanol for the hydrogen atom

Table 1.2 RATE CONSTANTS FOR INTERACTION OF $\bar{e}_{aq.}$, $\dot{H}$, $\dot{O}H$ (M^{-1} Sec^{-1})

COMPOUND	$\bar{e}_{aq.}$	$\dot{H}$	$\dot{O}H$
H_2	-	-	4.5×10^7
O_2	1.9×10^{10}	2.2×10^{10}	-
Methanol	1.0×10^4	1.6×10^6	5.0×10^8
2-propanol	-	6.5×10^7	1.5×10^9

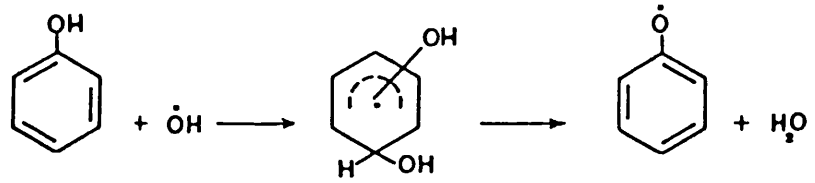
produced was noted as:

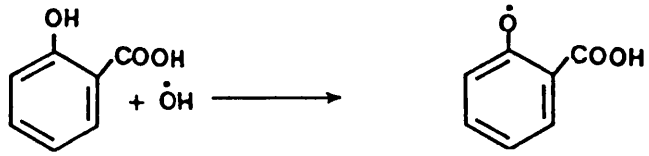
$$\dot{H} + O_2 \longrightarrow \dot{H}O_2$$

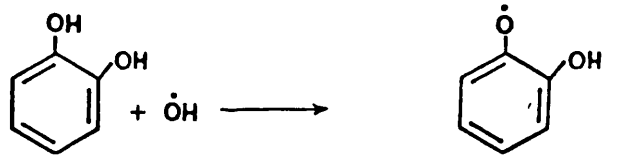
$$\dot{H} + CH_3CH_2OH \longrightarrow H_2 + CH_3\dot{C}HOH$$

Reactions of Radiolytic Products of Water With Organic Compounds Containing an Aromatic Ring

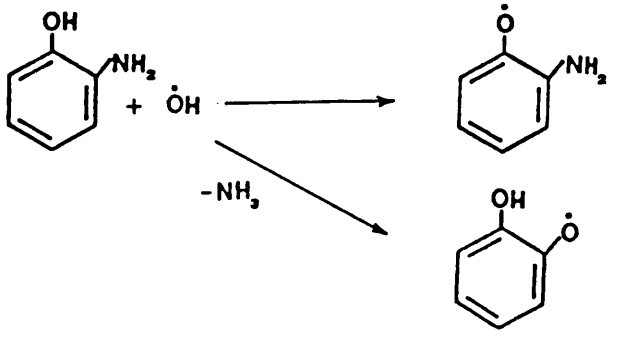
The reactions of the hydroxyl free radical with simple organic molecules such as phenol, substituted phenols and aniline have been studied[46,47,48] and the organic radicals produced were observed to be of the phenoxyl type formed by the additions of the $\dot{OH}$ to the aromatic ring.



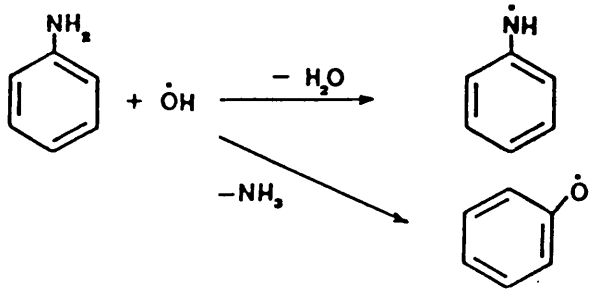


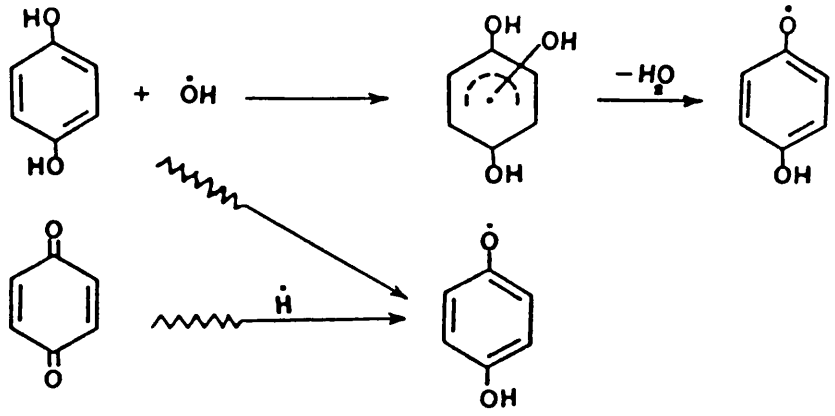


Semiquinone Radical

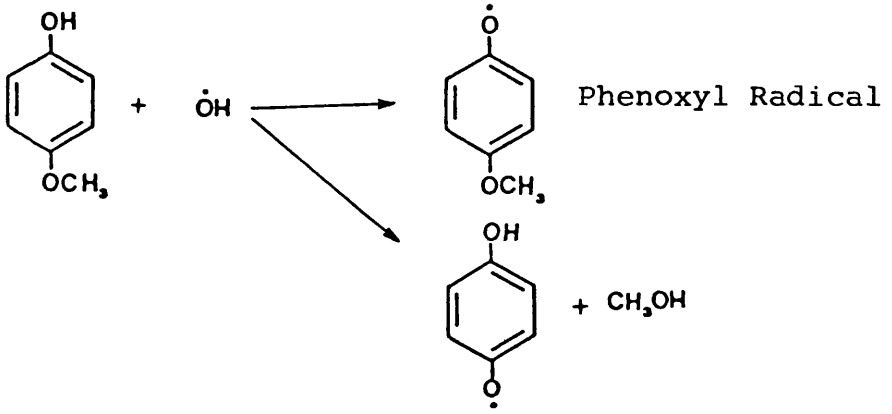


Aminophenoxyl Radical

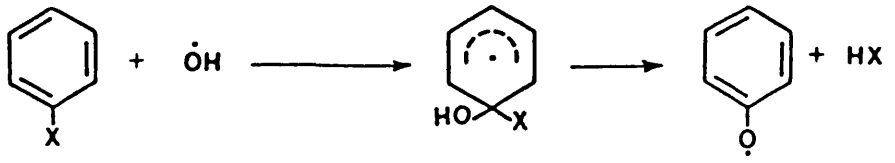

NH₂
+ ȮH
– H₂O
–NH₃
ṄH
Ȯ


HO
HO
+ ȮH
OH
OH
OH
– HO₂
Ȯ
OH
O
O
Ḣ
Ȯ
OH

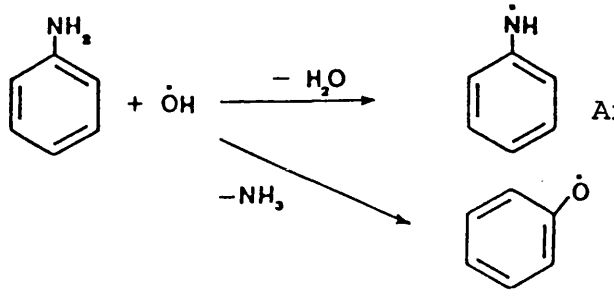
In the case of Methoxyphenol, the position of the hydroxyl group related to the methoxyl one greatly affected the yield, i.e. a high yield was obtained when they are ortho or para to each other and lower yield when in the meta position. This is due to the activation of the position of attack by the electron-donating property of either -OH or CH_3O- groups[49].



Generally, oxidative replacement of substituent "x" by a hydroxyl radical can be illustrated by the following reaction resulting in first an addition to the aromatic ring followed by abstration of a hydrogen:

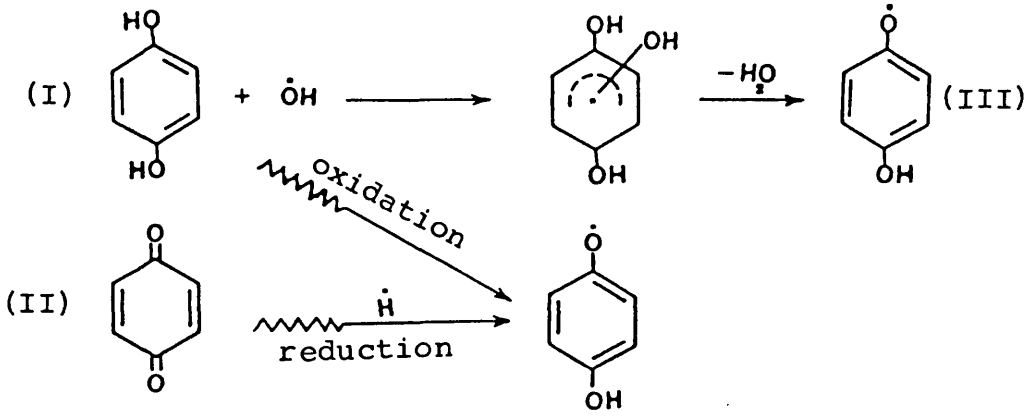


X = F or NO_2 or NH_2 [46,50]



Anilino Radical

phenoxyl Radical



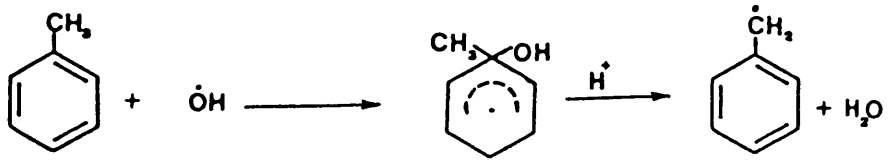
I - Hydroquinone

II - Benzoquinone

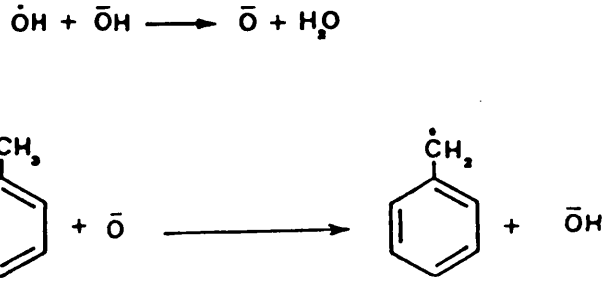
III - Benzosemiquinone

Pulse radiolysis of toluene and aromatic compounds containing a side chain such as benzyl chloride, bromide or acetate has shown that the hydroxyl radicals abstract preferentially the hydrogen atom from the side chain[51], for example:

a) In acid medium



b) In alkaline medium



The interaction of hydrated electrons with phenylalanine and related compounds has been studied by Hayon[52] who concluded that both deamination and addition to the aromatic ring occurred.

$$\bar{e}_{aq.} + ph\text{-}CH_2\text{-}CH(\overset{+}{N}H_3)C\bar{O}_2$$

addition to ring ← ph-$CH_2\dot{C}HC\bar{O}_2$ + NH_3

The deamination reaction has also been supported by Allen[53], who produced $ph\dot{C}H\bar{O}_2$ through dechlorination of α-chlorophenyl acetic acid through reaction with hydrated electron

$$\bar{e}_{aq.} + phCH(Cl)C\bar{O}_2 \longrightarrow ph\dot{C}HC\bar{O}_2 + Cl^-$$

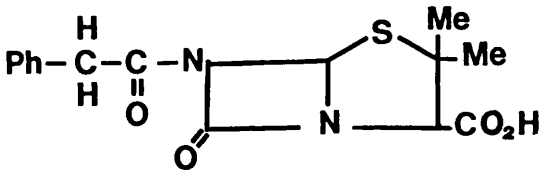
Ionising Radiation Effects on Pharmaceuticals

The feasibility of sterilising barbiturates in powder form by γ-irradiation was investigated by Jacobs et al[7] who found that some derivatives such as barbitone, phenobarbitone and phenobarbitone sodium were not affected by irradiation, while others such as pentobarbitone, pentobarbitone sodium and quinalbarbitone showed degradation products but at minimal concentrations.

The effect of γ-irradiation on several sulphonamides, in aqueous solutions and in the solid state, has been studied by Phillips et al[4,8] according to whom all the degradation effects were initiated by a reaction of the hydrated electron with the drug. However, the hydroxyl radical also initiated degradation in some sulphonamide forming an adduct by addition to the aromatic ring. The G^- value in aqueous solution varied from 3.5 - 5.1, while in solid state from

0.15 - 0.6 at a dose of irradiation of 2.5 M.rad.

The use of gamma irradiation for sterilisation of antibiotics has also been evaluated[5]. For example, the degradation pathways for penicillin G, neomycin, novobiocin and dihydrostreptomycin have been identified, and found to be similar to those commonly encountered when the antibiotics were subjected to acidic, basic, hydrolytic or oxidative treatments. No radiolytic products unique to γ-irradiation have been identified. For example, it was found that both the hydrated electron and the hydroxyl radical were responsible for the degradation of aqueous solutions of benzyl penicillin, where the hydroxyl radical reacted with the benzene ring of the side chain and induced cleavage of theβ-lactam bond resulting in formation of benzyl penilloic acid. Similarly the hydrated electron attacks the side chain resulting in the formation of benzyl penillic acid.



Benzyl Penicillin

However, tetracycline hydrochloride powder showed no degradation up to 2.5 M.rad, the dose that is sufficient for sterilisation[54].

Chang et al[55] subjected a number of selected colourants to doses of γ-radiation up to 2.5M.rad and no physical or chemical changes were observed, as shown by TLC, IR, visible and u.v. spectra.

The irradiation of a number of steroids in a dry state has shown that they are stable up to the sterilising dose of 2.5M.rad. However, in aqueous solutions, many steroids have been reported to be sensitive to ionising radiation due to the attack by the radiolytic products of water. For example, Allinson et al[56] have reported that, on irradiation of deaerated aqueous solutions of cortisone and deoxy-corticosterone, the following reactions may take place at different locations on the steroid molecule:-

Reactions with ring A

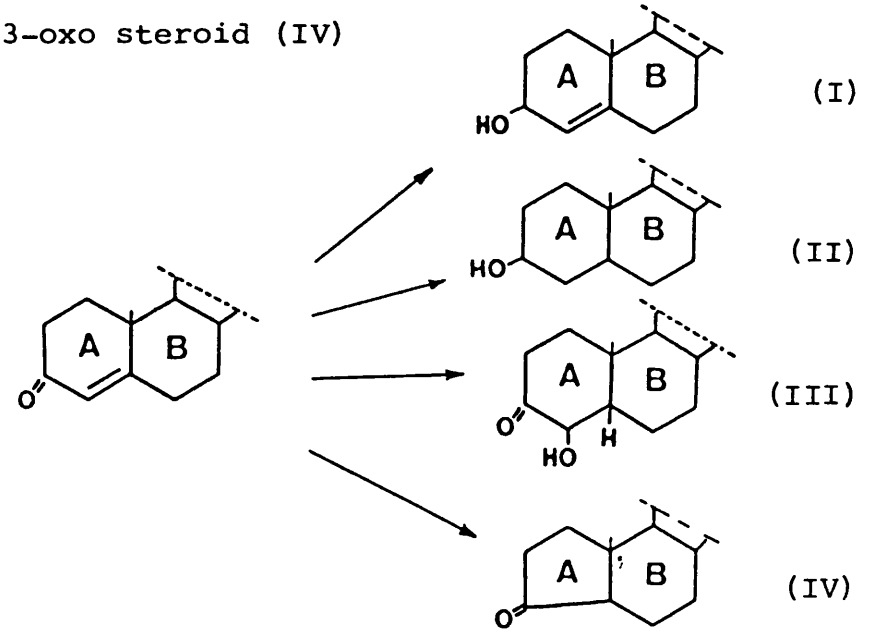
A series of reactions may take place at ring A as shown as follows in scheme (A).

i) reduction of the 3-oxo group. (I)

ii) complete reduction of the Δ^4-3-oxo system to give 3-β-hydroxy derivative (II)

iii) addition of one hydroxyl group and one hydrogen atom to the double bond (III)

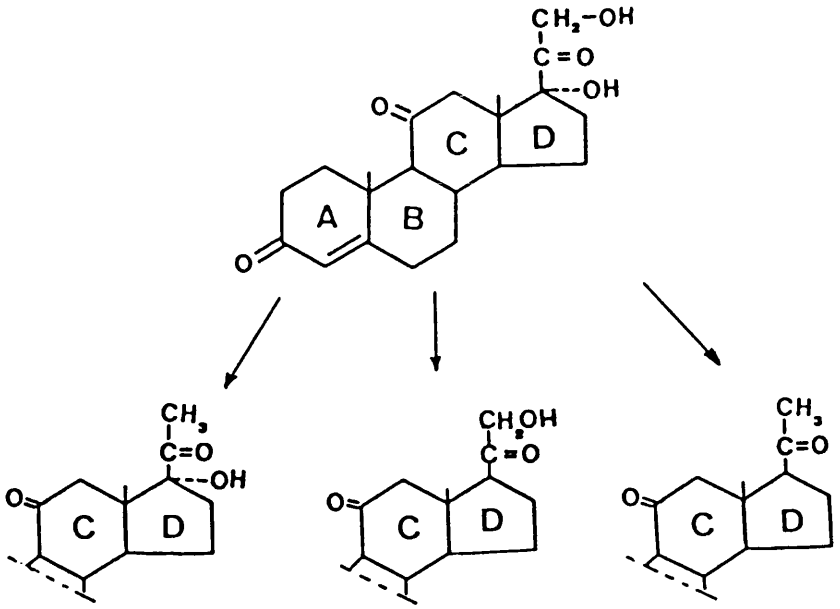
iv) elimination of a carbon atom from ring A to give A-nor-3-oxo steroid (IV)



Scheme (A)

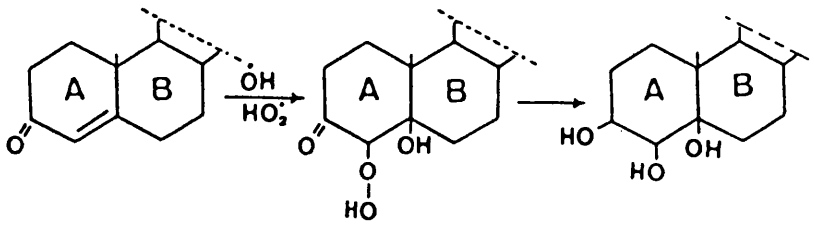
Side Chain Reactions on Ring D

Dehydroxylation in the C-17 or in the C-21 position of cortisone may also take place, as shown in the scheme (B) leading to the following reactions:-



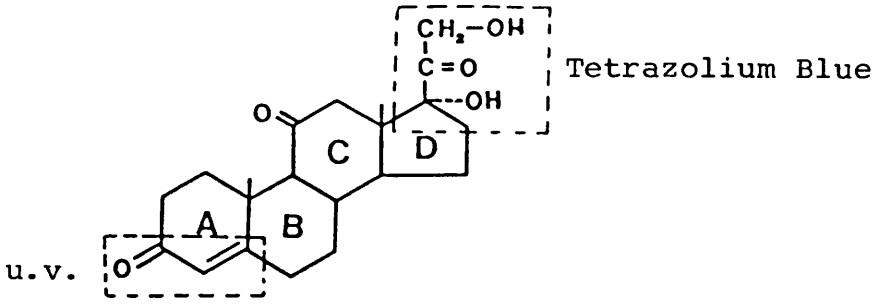
Scheme (B)

Further studies have been done by Scholes[57] on the radiolysis of aqueous solution of cortisone and deoxycorticosterone. He concluded that hydroxyl and hydroperoxy radicals could attack the double bond of ring A.

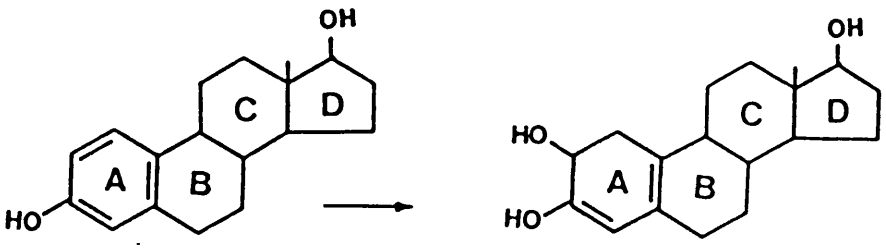


This type of structure was supported by the disappearance of the Ultraviolet absorption associated with the chromophoric α,β unsaturated Ketone grouping in ring A.

In addition to the hydroperoxide reaction on ring A, a second reaction involving the cleavage of the dihydroxy-acetone side chain may take place associated with a correspondin decrease in the tetrazolium blue reaction.



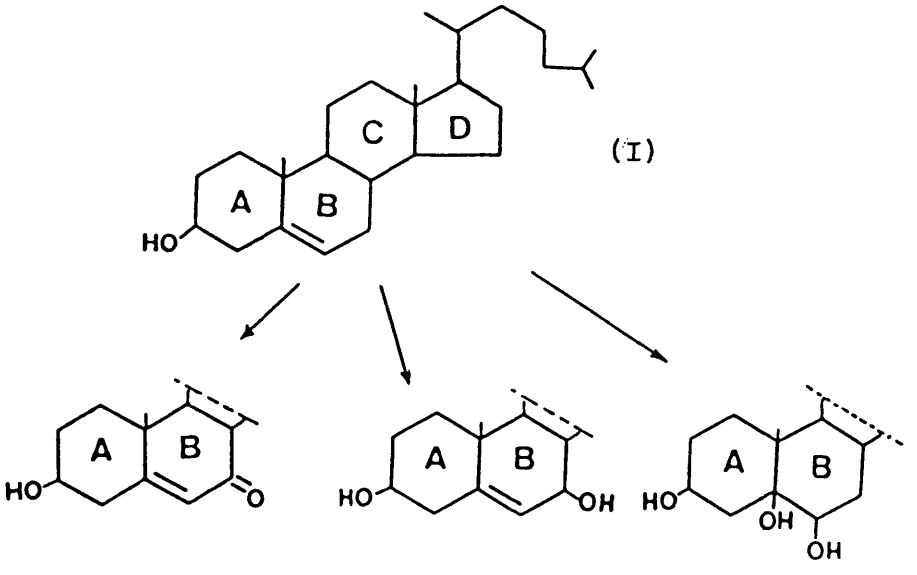
A similar addition of the hydroxyl group was also reported on gamma-radiolysis of estrone and estradiol in 1N sod, hydroxide. The products were 2-hydroxyestrone and 2-hydroxyestradiol respectively[58].



Estradiol 2-Hydroxyestradiol

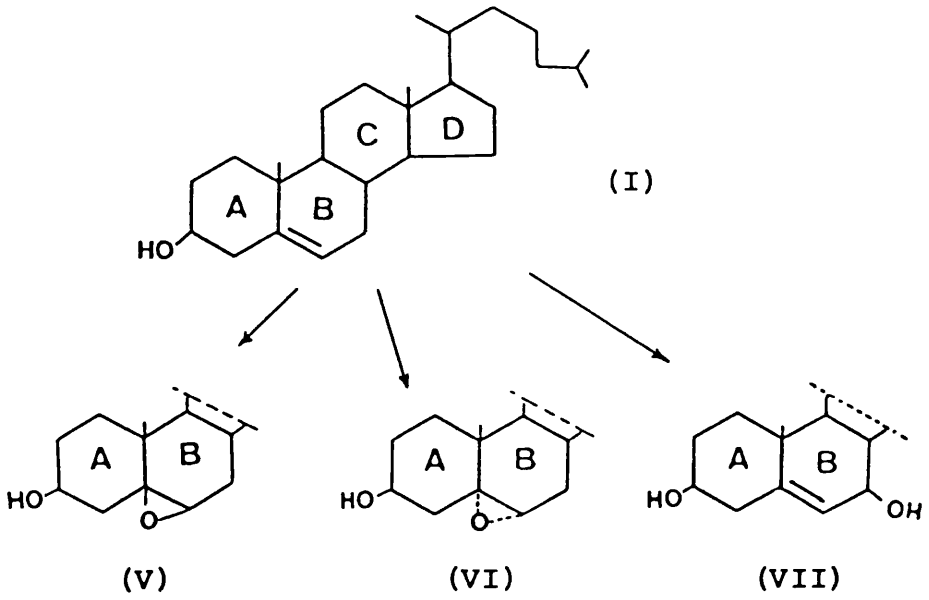
Therefore, the presence and position of a double bond in a steroid molecule as well as the presence of oxygen during irradiation can influence the possible resulting degradation products.

In organic solvents, steroids are more stable than in aqueous solutions when subjected to ionising radiation. The presence of functional groups in the steroid molecule increases its sensitivity to such radiation. When Cholesterol (I) was irradiated in methanol[59], attack at the double bond in the B-ring occurred producing 3-β-hydroxy-cholest-5-en-7-one (II), cholest-5-en-3β,7β diol (III) and cholestane-3β,5α,6β-trial (IV).



(I) (II) (III) (IV)

However, when irradiated in acetone or dioxane, cholesterol produced 5α,6β-epoxy cholestan-3β-ol (V) and 5β, 6β-epoxy cholestan-3β-ol (VI) as well as cholest-5-ene-3β,7β diol (VII).



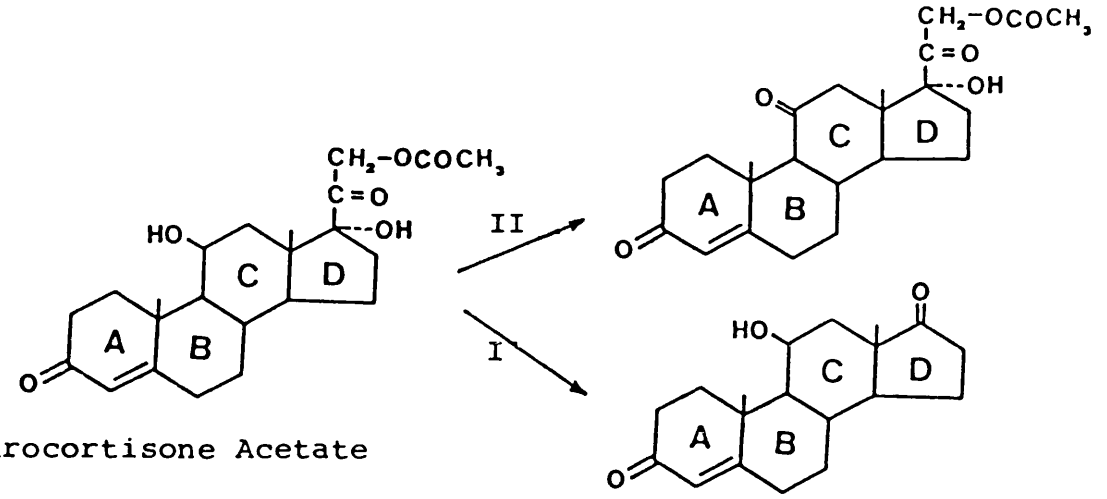
Hydrocortisone acetate, isoflupredone acetate, methyl prednisolone acetate and prednisolone were sterilised by Bussey et al[2] using Cobalt-60 irradiation. The minimum irradiation dose they found for sterilisation was 1.33 M rad. The degree of degradation indicated that a potency loss of one per cent or less could be expected. Similar results have been obtained by Kane et al[3] who showed that two major types of radiolytic degradation schemes were found to occur by the Cobalt-60 irradiation of corticosteroids:

I. the loss of the corticosteroid side chain on the D-ring to produce the C-17 Ketone.

II. the conversion of the C-11 alcohol to the C-11 Ketone.

Minor degradation products, derived from other changes affecting the side chain, were also identified in several

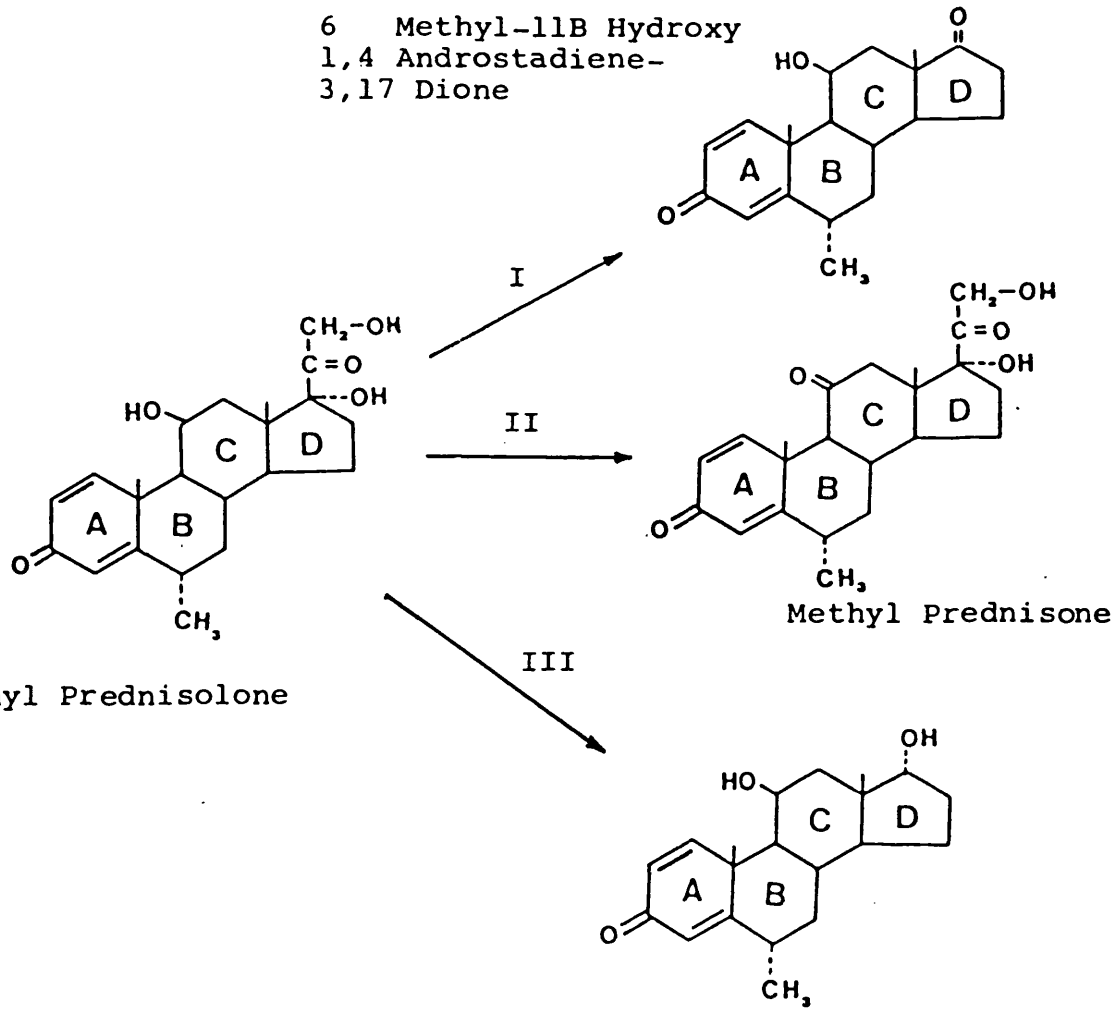
Cortisone Acetate



Hydrocortisone Acetate

11B Hydroxy 4-Androstene
3,17 Dione

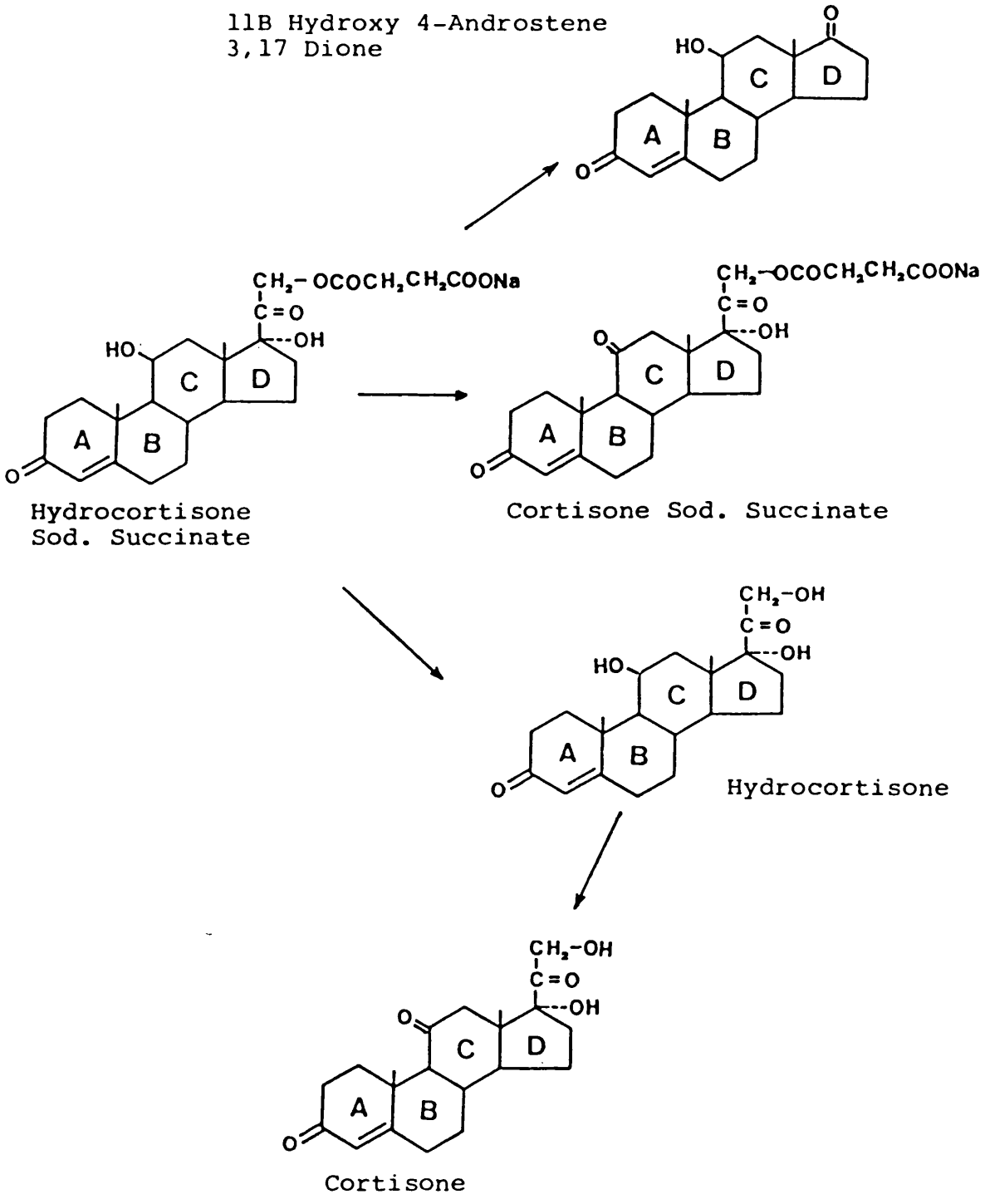
6 Methyl-11B Hydroxy
1,4 Androstadiene-
3,17 Dione



Methyl Prednisone

Methyl Prednisolone

11B, 17 Dihydroxy-6 Methyl
1,4 Androstadiene-3one

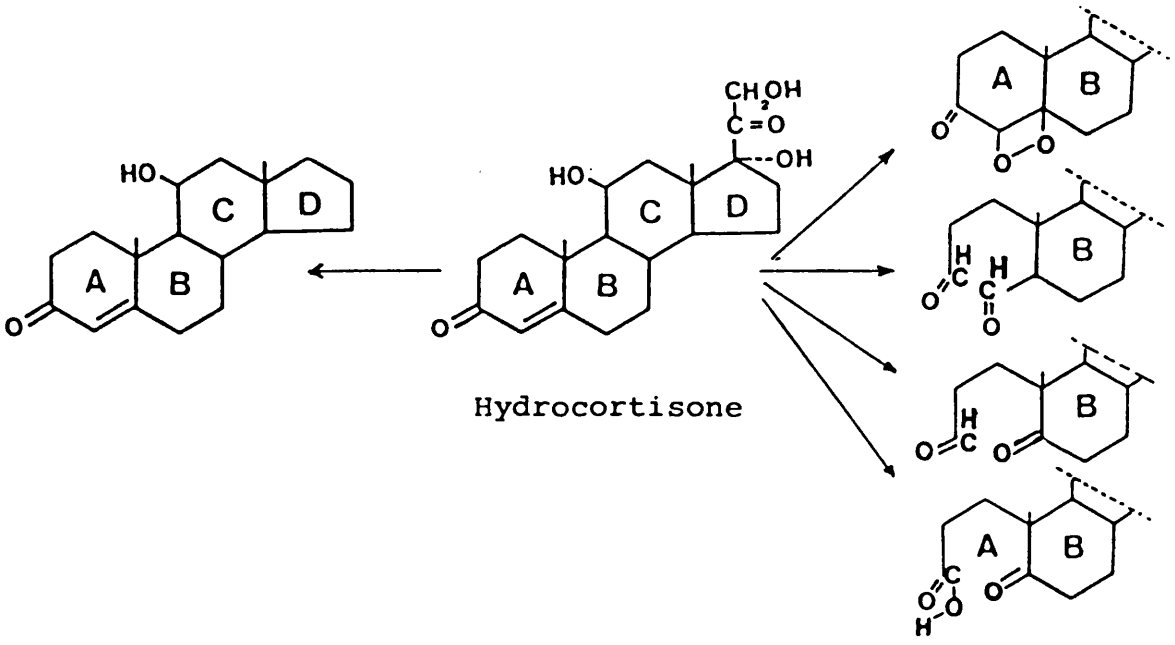


Cobalt-60 Radiolytic Degradation Pathways of Hydrocortisone Acetate, Hydrocortisone Sodium Succinate and Methyl Prednisolone

corticosteroids. For example, the loss of the C-17 side chain in methyl prednisolone was found to result in the formation of the C-17α alcohol in addition to the C-17 Ketone. Also, hydrocortisone was identified as an additional degradation product of hydrocortisone sodium succinate.

Stability of Pharmaceutical Preparations Containing Steroids

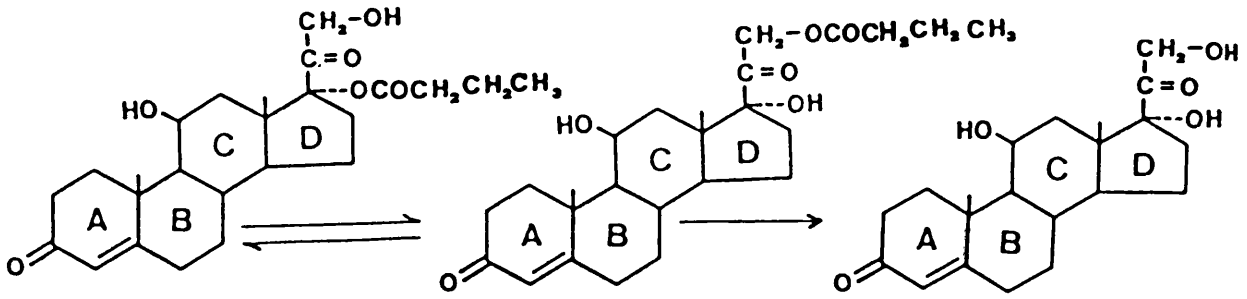
It is important when studying the radiation sensitivity of steroids to appreciate the possibility of thermal degradation of such compounds. For example the stability of hydrocortisone in various types of vehicles such as aqueous solution, polyethylene glycol ointment bases and oil in water or water in oil type emulsions, has been investigated[60,61,62]. The hydrocortisone was found to be very unstable in water and in polyethylene glycol ointment base because of an apparent attack on the C-17 side chain[60] or on ring A[62].



Hydrocortisone

Gupta[61] also reported that the addition of alcohol and glycerin to water had a stabilising effect.

Hydrocortisone butyrate, being more active biologically than the parent alcohol and having a better delivery to the site of action when topically applied[63], was subjected to stability investigations in semiaqueous (50%v/v propylene glycol in water) and formulated gel systems[64,65]. It was found that the C-17 hydrocortisone butyrate underwent reversible isomerisation to the more chemically stable C-21 ester which is unfortunately less active topically. The C-21 ester then hydrolysed to hydrocortisone, which in turn degraded to a complex mixture of compounds. This step was apparently catalysed by metal ions which would be inhibited by the addition of the chelating agent EDTA (disodium ethylene dinitrilotetra acetate[64].



Hydrocortisone 17-butyrate

Hydrocortisone 21-butyrate

Hydrocortisone

The degradation of hydrocortisone hemisuccinate has also been studied[66,67,68] at elevated temperatures and at different pH values. The blue tetrazolium assay confirmed that species devoid of the 17-dihydroxyacetone side chain were produced

subsequent to the formation of the steroid alcohol.

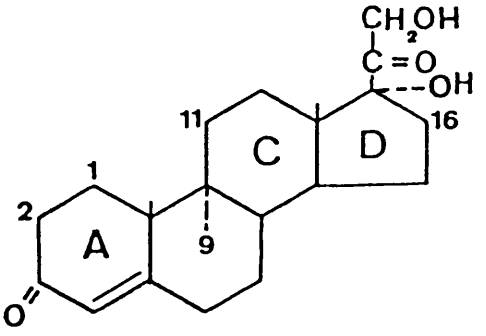
The complete thermal decomposition of buffered aqueous solution of fluprednisolone acetate was observed[69,70], and the free alcohol, formed, underwent further degradation.

In an organic medium, containing 50% camphor, 25% m-cresylacetate and 25% P-chlorophenol, which is a formula of a pulp-capping agent to reduce thermal sensitivity in dental restorations, the stability of prednisolone was studied by Bowles[71] who found that the steroid was stable for 3-5 years. The same worker also found that prednisolone in the anhydrous form was more stable in liquid paraffin than in water.

The stability of triamcinolone acetonide in water-ethanol solutions of varying pH in different buffer concentrations and ionic strength, and in polyethylene glycol ointment base has been studied[72]. The degradation was shown to be first-order with the same decomposition pathways as hydrocortisone i.e. one pathway through the attack on the ring A, and another through an attack on the C-17 side chain.

The Kinetics of the anaerobic decomposition of a number of corticosteroids, possessing a dihydroxyacetone side chain, were studied at various pH values at 100°C by Dekker et al[73]. At pH below 4.5, the hydroxyl group present at C-11 of hydrocortisone decreased the rate of decomposition as compared to the carbonyl group at the C-11 of cortisone. The introduction of a Δ^{-1} double bond in the hydrocortisone to produce prednisolone did not influence its anaerobic decomposition. The same worker also showed that dexamethasone was more stable than prednisolone, which means that the α-methyl group at

C-16 of dexamethasone increased its stability (as F-9 has no effect on the stability). Also, the orientation of the C-16 methyl group was an important factor in the stability of the steroid molecule, i.e. dexamethasone and betamethasone.



STEROID	CARBON ATOM NUMBER			
	1-2	9	11	16
Hydrocortisone	Saturated	α-H	β-OH	-H
Cortisone	Saturated	α-H	=O	-H
Prednisolone	Unsaturated	α-H	β-OH	-H
Betamethasone	Unsaturated	α-F	β-OH	β-CH_3
Dexamethasone	Unsaturated	α-F	β-OH	α-CH_3

Mechanisms of Protection in Radiolysis of Organic Systems

The addition of relatively small amounts of certain substances to organic systems can markedly reduce the yields of radiolysis products. The mechanisms of this protection can be broadly classified into two types[74]:

I. A scavenging effect of the additives on free radicals produced from primarily excited and ionised solvent molecules.

II. An effect of additives on the precursors of the first chemical decomposition.

Generally, a typical scavenger may involve both mechanisms at the same time.

Classification of Protection Mechanisms:

1 - Energy Transfer or Sponge Type Mechanism

If an additive has an excited or an ionised state lying energetically lower than those of the solvent, the energy transfer from the solvent to the additive may occur either via emission of a photon or by charge transfer[74]. Thus an additive of higher radiation stability than the solvent would exhibit a protective action. An illustrative example of this mechanism is the irradiation of glucuronic acid complexed with the additive P-toluidine-ammonium, where the degradation of the glucuronic acid, due to $\dot{O}H$ attack decreases due to energy transfer through the complex to the additive moiety[74].

2 - Quenching Mechanism:

When the additive cannot trap the electronic excitation energy of a single molecule, it may alternatively promote distribution of the initially localised energy among vibrational-rotational degrees of freedom of the neighbouring solvent molecules. Examples of additives of this type of mechanism are:

O_2, CH_3I, C_6H_5Br and I_2 [75,76].

3 - Negative-ion Formation Mechanism

The excited state, resultant from a simple neutralisation mechanism,

$$M^+ + \bar{e} \longrightarrow M^{\ddagger} \quad \ldots\ldots\ldots\ldots\ldots\ldots(1)$$

is generally higher than that produced by neutralisation involving a negative ion

$$M^+ + N^- \longrightarrow M^* + N \quad \ldots\ldots\ldots\ldots(2)$$

The negative ion N^- is formed by a "capture process" in which an added molecule (N) captures an electron before it can recombine with its positive hole (M^+). If $E(M^*)$ is sufficiently low, decomposition may be entirely prevented. This 'electron capture' by the additive may result in increasing the probability of ion-molecule reaction:

$$M^+ + M \longrightarrow \text{products} \quad \ldots\ldots\ldots\ldots(3)$$

However, the electron produced in the initial ionisation process by radiolysis may be captured by the parent positive hole as well as the molecular or ionic species[77,78],

so that the possible ion neutralisation processes may be represented not only by reaction (1) but also by the equation:

$$\bar{e} + M^+ + M \longrightarrow \text{products} \quad \ldots\ldots\ldots\ldots (4)$$

So when an additive is present which can capture an electron before it recombines with its positive hole, it interferes with the possible reaction (4) as well as the reaction (1). For example, when iodine is used as a protective in radiolysis of methanol it is necessary to postulate a quenching type action of iodine on excited states that would otherwise have rearranged to yield formaldehyde and hydrogen. Part of the observed yield of ethylene glycol is postulated to come from the reaction:

$$CH_3OH^+ + CH_3OH + \bar{e} \longrightarrow (CH_2OH)_2 + H_2$$

Iodine presumably also interferes with this process by electron capture mechanism.

4 - Ionisation Transfer and Ion-Molecule Reactions

When an additive (B) is present it can capture the ionisation before neutralisation can occur:

$$M^+ + B \longrightarrow M + B^+ \quad \ldots\ldots\ldots\ldots\ldots\ldots (5)$$

The subsequent molecule-ion electron reaction involves M,B^+ and the electron and the energy available for excitation and resultant chemical reactions can be greatly reduced. Thus the neutralisation process can be written

$$\bar{e} + (M^+ + B) \longrightarrow M' + B' \quad \ldots\ldots\ldots\ldots (6)$$

Where M' represents a state of lower energy than $M^{\ddagger}$ in reaction (1) and B' is in excited state of B which may not decompose.

A number of evidences now exist that energy transfer plays a significant role in the radiation chemistry of liquids. When transfer is to a low concentration additive, protection of the solvent occurs, occasionally at the sacrifice of the additive but, in many cases, without damage to either solvent or additive.

In the present work, it was decided to apply some of these protective mechanisms to investigate their effect on the sensitivity of the corticosteroids to γ-radiation.

Surfactants, Their Nature and Reactivity

Surfactants (surface active agents) are substances which, when present at low concentration in a system; have the property of adsorbing onto the surfaces or interfaces of the system and of altering to a marked degree the surface or interfacial free energies of those surfaces.

Surfactants have a characteristic molecular structure consisting of a structural group that has very little attraction for the solvent, known as a lyophobic group, together with a group that has strong attraction for the solvent, called the lyophilic group. This is known as an "amphipathic" structure. When a surfactant is dissolved in a solvent, the presence of the lyophobic group in the interior of the solvent causes a distortion of the solvent liquid structure, increasing the free energy of the system. In an aqueous solution of a surfactant this distortion of the water by the lyophobic (hydrophobic) group of the surfactant and the resulting increase in the free energy of thesystem when it is dissolved, means that less work is needed to bring a surfactant molecule than a water molecule to the surface. The surfactant therefore concentrates at the interface. On the other hand, the presence of the lyophilic (hydrophilic) group prevents the surfactant from being expelled completely from the solvent as a separate phase, since that would require desolvation of the hydrophilic group. The amphipathic structure of the surfactant therefore causes not only concentration of

the surfactant at the surface and reduction of the surface tension of the solvent, but also orientation of the molecule at the surface with its hydrophilic group in the aqueous phase and its hydrophobic group oriented away from it[79].

The hydrophobic group is usually a long chain hydrocarbon residue, while the hydrophilic group is an ionic or highly polar group. Depending on the nature of the hydrophilic group, surfactants are classified as:-

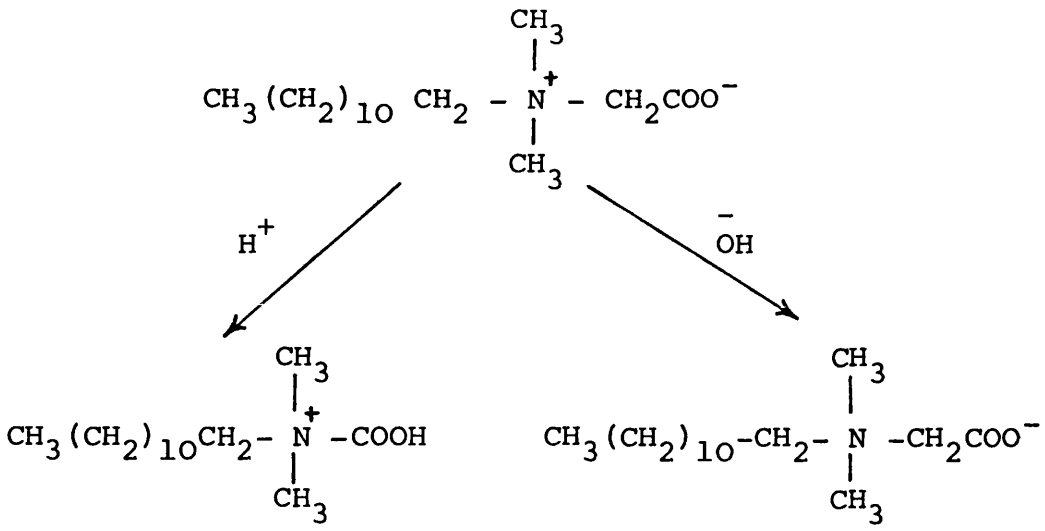
1 - Anionic: where the surface active portion of the molecule bears a negative charge which may be a carboxylic acid salt as in soaps, sulphonic acid salt as alkyl benzenesulphonate, sulphuric acid ester salt as sodium lauryl sulphate (NaLS) or phosphoric acid esters.

$CH_3(CH_2)_{11}SO_4^- Na^+$ (Sodium Lauryl Sulphate)

2 - Cationic: The surface active portion bears a positive charge. It may be a long chain amine, a diamine or quaternary ammonium salts such as quaternary ammonium chloride or trimethylammonium bromide (CTAB).

$CH_3(CH_2)_{15} N^+(CH_3)_3 B\bar{r}$

3 - Zwitterionic: both positive and negative charges may be present in the surface active portion (ampholytic). So it may be cationic, anionic or non-ionic in solution depending on whether the pH of the solution is above or below the isoelectric point of the molecules. For example, N-dodecyl N : N dimethylbetaine



(pH below isoelectric point)

(pH above isoelectric point)

4 - Non-Ionic: The surface active portion bears no apparent ionic charge which may be polyoxy-ethylenated alkylphenols, polyoxyethylenated straight chain alcohols or polyoxy-ethylenated polyoxypropylene glycols.

e.g. polyoxyethylene monohexadecyl ether (cetomacrogol)

$CH_3(CH_2)_{15}\ (OCH_2CH_2)_{21}\ OH$

Micellisation

In dilute aqueous solution, generally less than 10^{-4}M, the behaviour of ionic amphiphilic substances parallels that of strong electrolytes while the behaviour of non-ionic amphiphiles often resembles that of simple organic molecules.

At higher amphiphile concentrations, however, a pronounced deviation from ideal behaviour in dilute solution occur. Some of the physical properties which have been found to exhibit this type of behaviour are related to the interfacial tension, electric conductivity, the electromotive force, the pH, the density, the transport properties such as viscosity, and the optical and spectroscopic properties of the solution. The well defined changes in the physical properties are attributable to the association of the amphiphiles forming aggregates or micelles. The concentration at which the micelles appear is known as the critical micelle concentration or CMC. The micelles can be cationic, anionic, non-ionic or ampholytic depending on the chemical structure of the ionic surfactant and on the pH of the solution in the case of Zwitterionic surfactants. The critical micelle concentrations of non-ionic micelles are usually 100-fold smaller than those of ionic micelles containing comparable hydrophobic groups, and consequently, non-ionic micelles have higher micellar weights than ionic ones[80]. Important micellar structural differences also exist as a consequence of head group size and steric requirements. For example, since the positive charge residing on the quaternary nitrogen atom of cationic micelles is less exposed than the negative charge of anionic micelles, the proximity of the counterions to the head group is less in cationic micelles, and as a result their structure is more compact. Consequently, a cationic micelle is able to solubilise a larger quantity of a non-polar substrate than a similar molecular weight anionic micelle[81].

Micellar Size, Shape and Character

A schematic representation of an ionic spherical micelle is shown in fig. 1.1. Typically such micelles have average radii of 12-30 Å and contain 20-100 monomers. It is generally assumed that micelles at concentrations close to their CMC are roughly spherical[82,83]. The hydrophobic part of the aggregate forms the core of the micelle while the polar head groups are located at the micelle-water interface in contact with and hydrated by a number of water molecules. Some water molecules may be entrapped by the micelle[84,85,86] and under certain circumstances part of the hydrocarbon chain may extend into the aqueous phase[84]. The interior, or core, of the micelle has generally been inferred to be hydrocarbon-like from esr,nmr and spectroscopy[87,88].

The charged head groups and the relatively small counterions of the ionic micelle are located in a compact region, known as the Stern layer, which extends from the core to within a few angstroms of the shear surface of the micelle. The compactness of the stern layer is responsible for the reduction of the net charge on the micelle. Most of the counterions are however, located outside the shear surface in the Gouy-Chapman electrical double layer where they are completely dissociated from the charged aggregate and are able to exchange with ions in the bulk of the solution.

When the surfactant concentration markedly exceeds the CMC, the shape of the spherical or ellipsoidal micelle undergoes gradual changes. It elongates to assume cylindrical or lamellar structure[89]. Both ionic and nonionic solutes

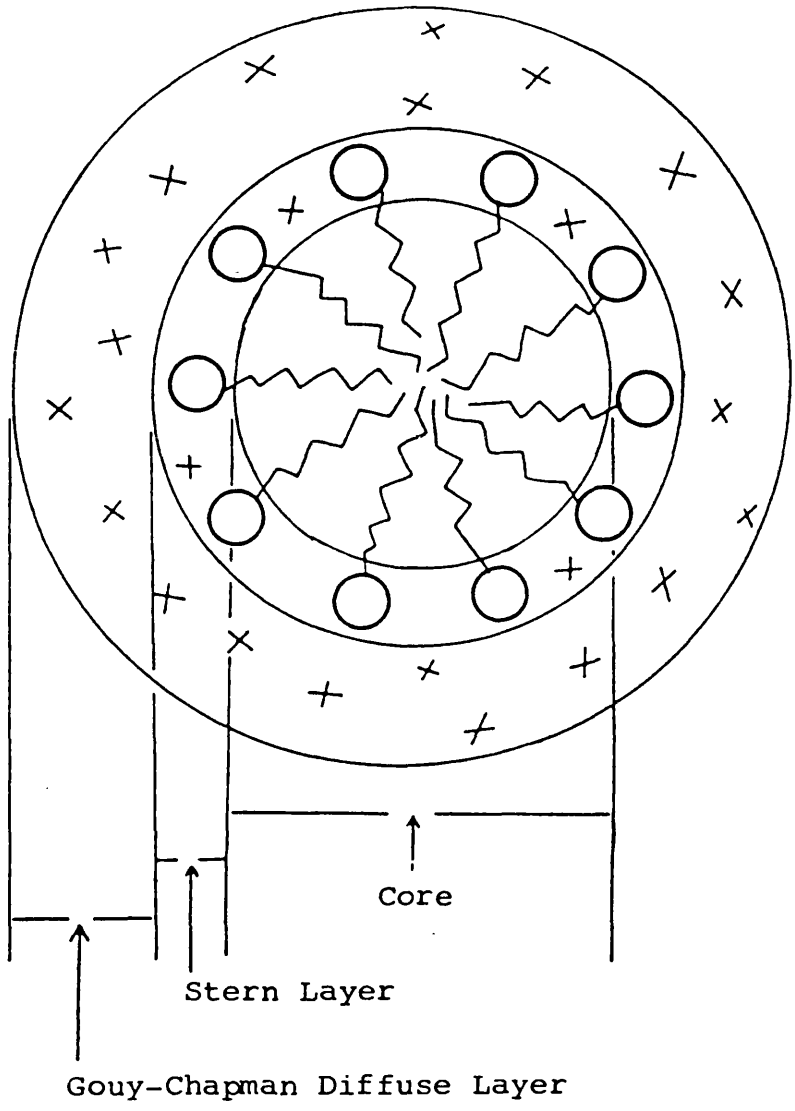
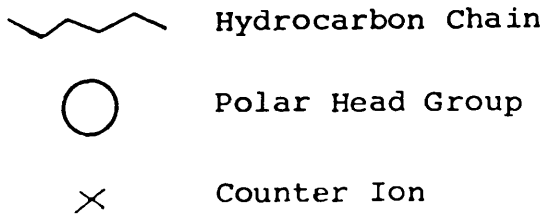


Fig. 1.1 Two-Dimensional Scheme of an Ionic Spherical Micelle



can, however, modify the micellar structure[90]. Such modifications generally consist of conversion of spherical or ellipsoidal micelles into elongated ones. A process of this type has been shown to lead to enforced counterion binding and consequently to pronounced changes in the micellar catalysis of certain reactions.

Factors Affecting the CMC and Micellar Size

1 - Nature of the Hydrophobic Group

For ionic surfactants an increase in the number of carbon atoms in unbranched hydrocarbon chains leads to a decrease in the CMC. The dependence of CMC on the number of carbon atoms (m) can be expressed by the empirical equation:

$$\log CMC = A - Bm$$

where A and B are constants for a homologous series. Accordingly the CMC is halved when the length of the straight hydrocarbon chain is increased by one methylene group. For chains of greater length than 16 carbon atoms this relationship no longer holds and further increase in the chain length often has no appreciable effect on the CMC, possibly due to the coiling of the long chains in the solution[91]. An even more pronounced decrease in CMC with an increase in hydrocarbon length is noted with non-ionic surfactants, where the addition of one methylene group causes the CMC to decrease to approximately one-third of its original value. For branched hydrocarbon chains the effect on the CMC of an increase in the number of carbon atoms in the branched segments of the chain is not as great as that following a similar increase when the carbon atoms are in a straight

chain. As expected, the increased hydrophobicity conferred by an increase of chain length also causes an increase in micellar size. Arnarson *et al*[92] have demonstrated a linear relationship between the aggregation numbers of polyoxyethylene non-ionic surfactants and the number of carbon atoms in the hydrocarbon chain.

2 - Nature of the Hydrophilic Group

There is a pronounced difference between the CMCs of ionic and non-ionic surfactants with identical hydrophobic moieties. The lower CMCs of the non-ionic surfactants are a consequence of the lack of electrical work necessary in forming the micelles. It has been reported by Anacker and Coworkers[93] that an important factor controlling the micellar size is the mean distance of closest approach of a counterion to the charge centre of the surfactant. For example, decylammonium bromide forms very much larger micelles than decyltrimethylammonium bromide. However, solvent interaction may be an influential factor[94]. For example, hydrogen bonding between the oxygen atom of decylmorpholinium bromide and water is thought to be responsible for the smaller micellar size of this compound as compared to decylpiperidinium bromide which does not interact with the solvent in this way.

For the polyoxyethylated ether type of non-ionic surfactant an increase in the length of the polyoxyethylene chain causes an increase in the CMC and a decrease in the micellar size. This may be because increasing the polyoxyethylene chain length makes the monomer more hydrophilic and results in an increase in the CMC. The same effect may

be partially responsible for the apparent decrease in micellar size.

3 - Effect of Additives

Numerous studies have been reported of the effects of added electrolyte on the micellar properties of ionic surfactants. Values of CMC in the presence of electrolyte are available in the reference text by Mukerjee and Mysels[95]. Addition of electrolyte causes a reduction in the thickness of the ionic atmosphere surrounding the polar head groups and a consequent decreased repulsion between them. These effects are manifest as a reduction in CMC and an increase in aggregation number. It has been reported[96,97] that the shape of the micelles of sodium lauryl sulphate (NaLS) undergoes a transition from sphere shape to rod-like shape at concentration of approximately 0.45M NaCl at 25°C and a corresponding dramatic increase of aggregation number at a similar electrolyte concentration takes place. Shinoda[98] has reported that solubilised hydrocarbon increases the micelle size and changes in the curvature of the micelle surface leading to a decrease in the CMC.

The effect of urea addition on the CMC is of interest in view of the disruptive effect which this compound has on the structure of water. The increase in the CMC of dodecylpyridinium iodide with an increase in urea concentration, as reported by Mukerjee[99], confirms the role of water structure in micelle formation. However, the effects of urea on the CMC are relatively small compared to hydrophobic effects.

A detailed study of the effect of inorganic additives on solutions of non-ionic surfactants has been reported by

Schott and Co-workers[100,101]. However, it has been noted that the lowering of the CMC of polyoxyethylated non-ionic surfactants following the addition of electrolyte is much smaller than electrolyte effects on ionic surfactants.

The addition of lower alcohols to ionic surfactants causes a decrease in the CMC which becomes more pronounced with increase in hydrophobicity of the added alcohol. The main factor which causes a decrease in CMC is likely to be the reduction of the free energy of the micelle due to the diluted surface charge density on the micelle. The effect of n-alcohols on the aggregation number of ionic surfactants have been discussed by Backlund *et al*[102] who found that water soluble alcohols such as methanol, ethanol and butanol are predominantly dissolved in the water phase and may increase or decrease the aggregation number depending on the alcohol concentration. Moderately soluble alcohols such as pentanol and hexanol are distributed between the aqueous and micellar phases and at low concentrations may increase the aggregation number. Sparingly soluble alcohols such as heptanol and octanol are almost entirely solubilised in the micelles and thereby increase the aggregation number of the surfactant.

Studying the effect of alcohols on the non-ionic surfactant systems, Green[103] reported that, whereas methanol and ethanol cause a CMC increase, the higher alcohols butanol and pentanol cause a decrease in this property. Propanol exhibits an intermediate effect, where low concentrations cause a decrease in CMC, higher concentrations (>1 mol.l^{-1})

cause an increase. The CMC increasing effect of the lower alcohols has been attributed to a weakening of the hydrophobic binding, while the CMC decreasing effects are thought to be a consequence of the penetration of the alcohols into the palisade layer of the micelle, forming a mixed micelle.

Reactivity in Surfactant Systems

Chemistry at Interfaces

Adsorption of substances at interfaces can lead to an ordering of molecules that is not encountered in the bulk solution. Menger[104] has illustrated the possibilities of reactions at interfaces by the investigation of the reaction of the water-insoluble ester p-nitrophenyl laurate in heptane with imidazole in an adjacent aqueous phase. Because of the insolubility of the ester in water, any reaction between the species must occur at the interface. It was shown by the same author that, where water-insoluble agents have to be reacted with water-soluble reagents, addition of an emulsifier can assist the reaction by increasing the surface area available for reaction[105].

Micellar Reactions

Many studies on micellar reactions have been carried out[106,107,108]. One of the most comprehensive studies has been carried out by Bruice *et al*[109] who determined the rate of alkaline hydrolysis of neutral, positively and negatively charged esters when incorporated into micelles of neutral, positive and negative charges. For all cases, the rate of hydrolysis was found to decrease with increasing concentrations of surfactant. The association of the esters

with the micelles must either decrease the availability of the ester to $\bar{O}H$ attack or provide a less favourable medium for the hydrolysis reaction to occur.

Reactions of $\bar{e}$aq., H$^{\cdot}$ and $\dot{O}H$ with Micelle-Forming Surfactants

Determination of the rate constants of the primary radicals generated in the radiolysis of water with the surfactants is a necessary prerequisite for investigating rates of reactions of micelle solubilised substrates with these radicals. Rate constants for the reactions of $\bar{e}$ aq., H$^{\cdot}$ and $\dot{O}H$ with different types of surfactants have been determined by many authors[13,110,111,112] and are shown in table 1.3. As expected, the reactivity of straight chain hydrocarbons of n-alkyl surfactants with the hydrated electron is very low. In negatively charged micelles such as NaLS, the surface potential is sufficient to repel $\bar{e}$ aq. by making reaction with substrates unlikely if not impossible[113]. In hexadecylpyridinium chloride (C PyCl) micelles where the positively charged head group is highly reactive with $\bar{e}$ aq., rate constants for $K_{\bar{e}aq.}$ + micelle of up to 5×10^{12} $M^{-1}Sec^{-1}$ have been observed[114].

From table 1.3 it is evident that the hydroxyl radical and hydrogen atom are more reactive towards the monomeric surfactants by factor of 10^2 to 10^3 than the hydrated electron. More significant is the fact that rate constants for the reactions of $\dot{O}H$ with surfactants are about an order of magnitude smaller above their critical micelle concentrations than below them.

Hydrogen atom reactivities also present an interesting picture. For the surfactants containing straight saturated

alkyl chains such as CTAB and NalS, the rate constants for hydrogen atom reactions are concentration independent. Conversely, the rate is concentration dependent for the non-ionic surfactant Igepal Co-730. The rate constant for the reaction of hydrogen atoms with this surfactant is some 5-fold smaller above the critical micelle concentration than below it.

Table 1.3 RATE CONSTANTS FOR THE REACTIONS OF $\bar{e}aq.$, $\dot{O}H$, $\dot{H}$ WITH SURFACTANTS

Surfactant	Reaction Rate Constant (1 $mole^{-1} Sec^{-1}$)		
	$\bar{e}aq.$	$\dot{O}H$	$\dot{H}$
CTAB			
$<$CMC	$\leqslant 9 \times 10^5$	1.04×10^{10}	1.6×10^8
$>$CMC	$< 1 \times 10^7$	2.1×10^9	1.6×10^8
NalS			
$<$CMC	$\leqslant 2 \times 10^5$	7.6×10^9	1.2×10^8
$>$CMC	$< 1 \times 10^7$	5.0×10^8	1.2×10^8
Polyoxyethylene(15) nonylphenol (Igepal CO-730)			
$<$CMC		1.1×10^{10}	2.1×10^9
$>$CMC	$\leqslant 1 \times 10^6$	1.7×10^9	4.9×10^8

It is likely that for these ionic surfactants hydrogen atom abstraction occurs, while in the case of Igepal Co-730, hydrogen atom addition to the aromatic ring is likely to occur. Hydrogen atom abstration from the ionic surfactants can occur at the CH_2 groups located near the micellar surface in which case the hydrogen atom must penetrate the micelle

only slightly. Also, there is evidence for protrusion of the hydrocarbon chains, at least to the stern layer, for these ionic micelles. On the other hand the aromatic moiety of Igepal Co-730 is likely to be located further from the micellar surface and protected by the polyoxyethylene palisade layer. Consequently, the hydrogen atom would need to penetrate the non-ionic micelle more deeply for hydrogen addition to occur[80]. As expected, the rate constants for the interaction of hydroxyl radical decreases with increasing micellar size[115]. Generally, the micellar form of surfactants is less reactive to the radiolytic products of water than the monomeric form.

Reactions of $\bar{e}aq.$, $\dot{H}$ and $\dot{O}H$ with Substrates in the Presence of Micellar Surfactants

Interactions of the primary reducing and oxidising species with surfactant solubilised aromatic hydrocarbons resulted in the observation of significant micellar effects. For example Fendler and Patterson[13] have reported a 3-fold decrease in the rate constant for electron addition to benzene occurred curvilinearly as a function of NaLS concentration while a notable increase of the rate constant was observed in the case of CTAB. The observed rate inhibition implies either that the rate of electron attachment is different in the micellar phase than in the bulk aqueous phase or that the penetration of $\bar{e}aq.$ to the site of solubilised benzene is hindered electrostatically by the negatively charged NaLS micellar surface. In CTAB, benzene is solubilised at the CTAB water interface[116], therefore rate enhancements

may be explained in terms of electrostatic interactions between the π electron system of the benzene molecule and the net positive charge on the CTAB micelles surface which are likely to render benzene more susceptible to nucleophilic attack by the electron. Similarly, rate constants for electron addition to benzophenone, benzoquinone, 2,5 dimethyl-benzoquinone and 2,6 dimethylbenzoquinone increase in the presence of micellar CTAB but decrease in micellar NaLS and igepal co-730 [80].

Micellar effects have also been observed on the products formed in the interaction of $\bar{e}aq.$, $\dot{O}H$ and $\dot{H}$ with uracil and thymine[117]. At surfactant concentrations below the CMC's the results obtained indicate the prevalance of competition between the surfactants and the pyrimidines for the radiolytic species. However, at the surfactant concentrations above the CMC's, the values obtained are smaller than expected, indicating that marked micellar effects result when the monomeric surfactants aggregate to form micelles. Another important observation is that the micellar effects in the radiolysis of thymine and uracil are very similar for the three surfactants used: non-ionic Igepal Co-730, anionic NaLS, and cationic Cetyltrimethylammonium chloride (CTACl) indicating that the electrostatic effects are not the predominant factor. Also the decrease in the yield of thymine dimers in all of the systems in the presence of micelles is more pronounced than the decrease obtained for uracil dimers under the same conditions. These results indicate that solubilisation of the intermediates in the micellar phase contributes to the observed

micellar effect on the stability of solubilised material.

Stability of Drugs in Surfactant Systems

Many investigations have been carried out on the mechanisms of catalysis and inhibition of breakdown of drugs in surfactant systems.

The Kinetics of alkaline hydrolysis of indomethacin in non-ionic surfactant systems have been investigated[118]. Protection is observed, in contrast to the effect of a cationic surfactant which increases the hydrolysis of the drug. Selection of a suitable concentration of polysorbate 80 and adjustment of solutions to pH 4.6 reduced to half the autoxidative degradation of methylprednisolone even in the presence of oxygen[119]. Contrary to electrostatic theories of stabilisation, the base catalysed hydrolysis of procaine was inhibited by non-ionic, anionic and cationic micelles although procaine was found to be located in the outer layers of CTAB and N-dodecylbetaine[120].

In acidic media, cationic and non-ionic surfactants reduced the degradation of several penicillins by a factor of 4 to 12 while anionic surfactant increased the rate[121]. In contrast to the penicillins, the acid degradation of cefazolin, a relatively acid-unstable cephalosporin, was not influenced by the presence of any of the surfactants suggesting that this antibiotic is not sufficiently bound to any of the surfactant micelles.

Current interest in radiation sterilisation of pharmaceuticals led Fletcher and Davies[122] to investigate the sensitivity of benzocaine as a model drug to irradiation in

aqueous solution. Cetrimide and polysorbate 80 appeared to protect the drug from the deleterious effect of a Cobalt-60 source at doses up to 0.3 M.rad. This investigation has been extended by Chingpaisal[12] who studied the stability of benzocaine in aqueous solution in the presence of the cationic and anionic surfactants, CTAB and NaLS respectively and in formulated creams when they were exposed to gamma-radiation.

The interpretation of the mechanisms of protection against gamma rays may not always be straight forward, for AL-Saden et al[123] have found that gamma irradiation of non-ionic surfactant solutions can lead to polyoxyethylene chain scission. This in turn leads to the formation of mixed micelles between the surfactant and the more hydrophobic degraded species.

It is clear that the aqueous solutions of the drugs in the presence of different types of surfactants either below or above their CMC are complicated systems, and therefore in the investigation of the degradation caused by gamma-radiation of these drugs, many factors such as the type of surfactant, its concentration, the pH of the solution, the presence of electrolytes and the polarity of the drug should be taken into consideration.

2. MATERIALS AND GENERAL METHODS

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CHEMICALS

Acetonitrile (HPLC grade)	Fisons Scientific Apparatus Ltd.
Cetomacrogol 1000 BP	Evans Medical Ltd.
Cetomacrogol Emulsifying Wax BP	J. M. Loveridge Ltd. (Southampton)
Cetyl Alcohol (PFS, Research Laboratory grade)	Sigma Chemical Company
Cetyl trimethyl ammonium bromide BP	BDH Chemicals Ltd.
Chlorocresol BP	Evans Medical Ltd.
Deoxycorticosterone (PFS Research Laboratory grade)	Sigma Chemical Company
Ferrous Ammonium Sulphate (Analar grade)	BDH Chemicals Ltd.
Glycerol (PFS, Research Laboratory grade)	Sigma Chemical Company
Hydrocortisone (PFS, Research Laboratory grade)	Sigma Chemical Company
Hydrocortisone-21 Acetate (PFS, Research Laboratory grade)	Sigma Chemical Company
Hydrocortisone 21- disodium phosphate (PFS, Research Laboratory grade)	Sigma Chemical Company
Iodine (SLR)	Fisons Scientific Apparatus Ltd.
Liquid Paraffin BP	Evans Medical Ltd.
Methanol (HPLC grade)	Fisons Scientific Apparatus Ltd.
Potassium dihydrogen phosphate (Analar grade)	Fisons Scientific Apparatus Ltd.
Potassium iodide (Analar grade)	BDH Chemicals Ltd.
Prednisolone 21- Sodium Succinate (PFS, Research Laboratory grade)	Sigma Chemical Company

1,3 propanediol (Analar grade)	BDH Chemicals Ltd.
n-propanol (Analar grade)	BDH Chemicals Ltd.
2-propanol (Analar grade)	BDH Chemicals Ltd.
Propylene glycol (PFS, Research Laboratory grade)	Sigma Chemical Company
Sodium Chloride (Analar grade)	BDH Chemicals Ltd.,
Sodium hydroxide (Analar grade)	BDH Chemicals Ltd.
Sodium lauryl sulphate	Eastman Kodak Company
Sulphuric acid (Analar grade)	Fisons Scientific Apparatus Ltd.
Tetrazolium blue (Analar grade)	BDH Chemicals Ltd.
White Soft Paraffin BP	Evans Medical Ltd.

Gases

Oxygen, Nitrogen (oxygen free), Helium and nitrous oxide — Air Products Ltd.

Equipment

Volumetric glassware: "B" grade glass-ware was used.

Balance: A single pan balance model R20 manufactured by Oertling was used.

Cling Film: was used to seal the small vessels during irradiation of the dosimetric solution.

Irradiation vessels:

1- "Small irradiation vessels" used for irradiation solutions were made of pyrex glass and had an internal diameter of 7.5mm and external diameter of 10mm and a length of 70mm.

2- Irradiation vessels for ointment and cream were made of glass and had an internal diameter of 31mm, and external diameter of 34mm and a length of 60mm.

Spectrophotometer: A Unicam SP500 single beam UV/Vis. Spectrophotometer and a Perkin-Elmer-Lambda 3 UV/Vis Spectrophotometer were used with 5mm and 10mm quartz cells respectively.

High Pressure Liquid Chromatography

A constametric IIIG Metering pump (LDC/Milton Roy) in conjunction with a LDC/Milton Roy UV/Vis variable wavelength detector and a recorder were used. Two stainless steel columns packed with the stationary phase of appropriate particle size were used throughout the work. One was 25 x 0.46cm, packed with spherisorb ODS of 10 microns particle size, while the other was 15 x 0.46cm packed with spherisorb ODS of 5 microns particle size. A 20 µl loop was used for the injection of the samples.

Thin Layer Chromatography

Silica gel GF plastic sheets as well as reverse-phase chromatoplates, containing U.V. indicator, of 0.2mm thickness (Anachem Ltd.) were used and developed in a glass tank 225m x 225mm x 75mm.

Preparative Thin Layer Chromatography

Silica gel GF Chromatoplates of 2mm thickness (Anachem Ltd.) were used.

Radiation Source

A. Gravatom Cobalt-60 Source: consists of four 15cm fixed rods of Cobalt-60 placed equidistant around the sample cage, in its lowered position, within a large lead container. The sample cage is 3.5cm in diameter and is made of stainless steel tubing with a 15cm section cut from one side to allow samples to be placed in it. The cage can be raised and lowered

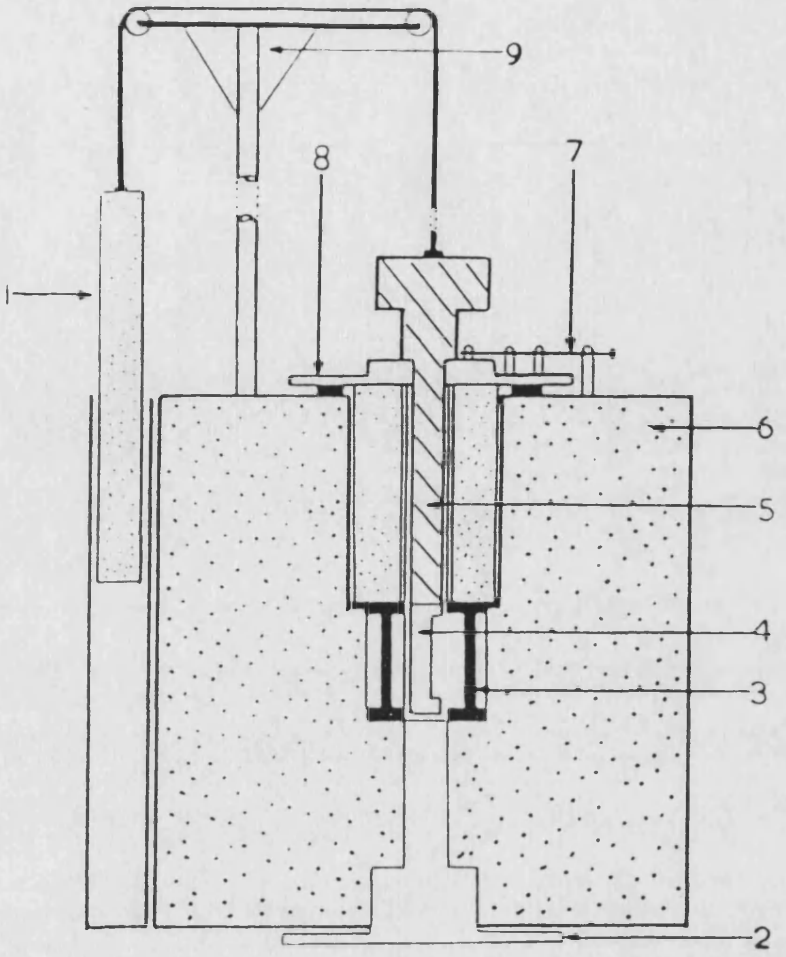


Fig. 2.1 Vertical Section of Gravatom Cobalt-60 Source

1. Counter Weight Assembly
2. Bottom Shield Assembly
3. Cobalt-60
4. Sample Cage
5. Central Plug Assembly
6. Lead Shielding
7. Locking Pin
8. Shielding Plus Assembly
9. Gantry Assembly

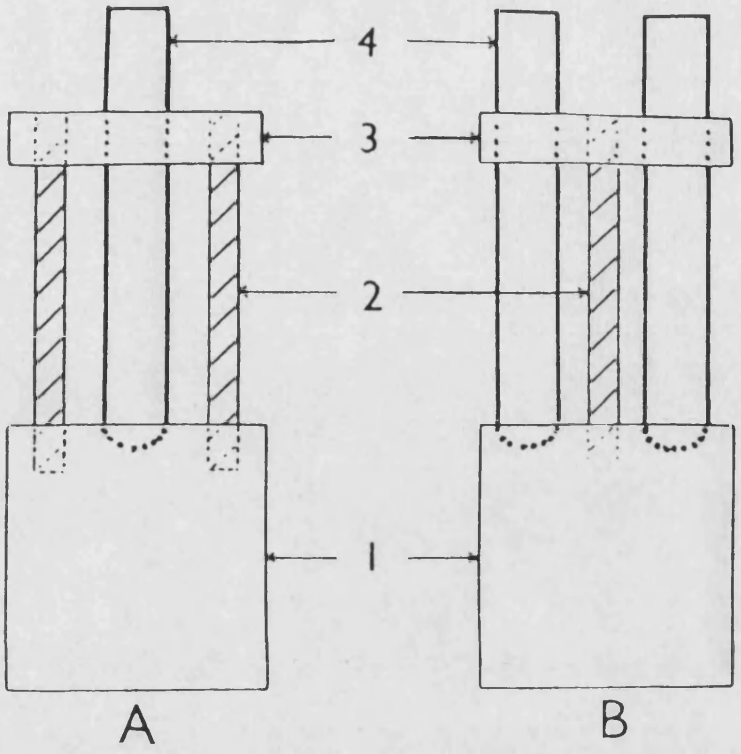


Fig. 2.2.a Side View of Irradiation Jigs

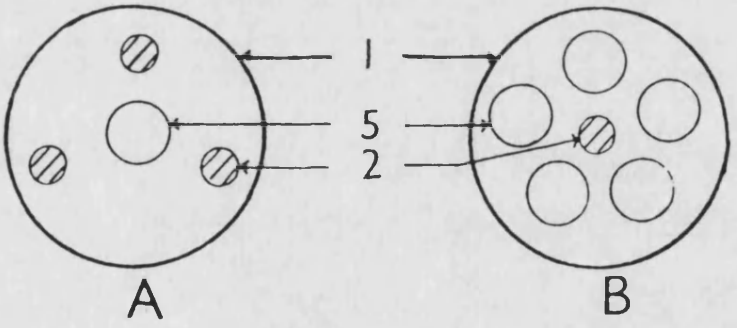


Fig. 2.2.b Plan View of Irradiation Jigs

A. Jig for Central Position in Sample Cage
B. Jig for Five Positions Round the Periphery of Sample Cage

1. Platform
2. Brass Rod
3. Support Platform
4. Small Irradiation Vessel
5. Position of Small Irradiation Vessel

by means of a pulley attached to a counter weight. Fig. 2.1.

Jig: when the small irradiation vessels were used, two types of jig could be used. One type for irradiating a single small vessel, and another type for irradiating five small vessels, fig. 2.2a, 2.2b.

B. Gravatom Caesium-137 Source: The irradiation unit, fig. 2.3a, has a vertical radiation beam with exposure chamber built from interlocking lead bricks. A lead door with a safety lock provides access to the chamber. The caesium-137 source is mounted such that it can be drawn horizontally into the exposure position by means of an electric drive. Exposure time can be controlled automatically using the "expose" - "return" buttons on the control panel.

Jig: When the small irradiation vessels were used, 10 vessels - jig made of plastic was used fig. 2.3b. The jig was placed on a platform which could be raised and lowered to the appropriate distance from the radiation source.

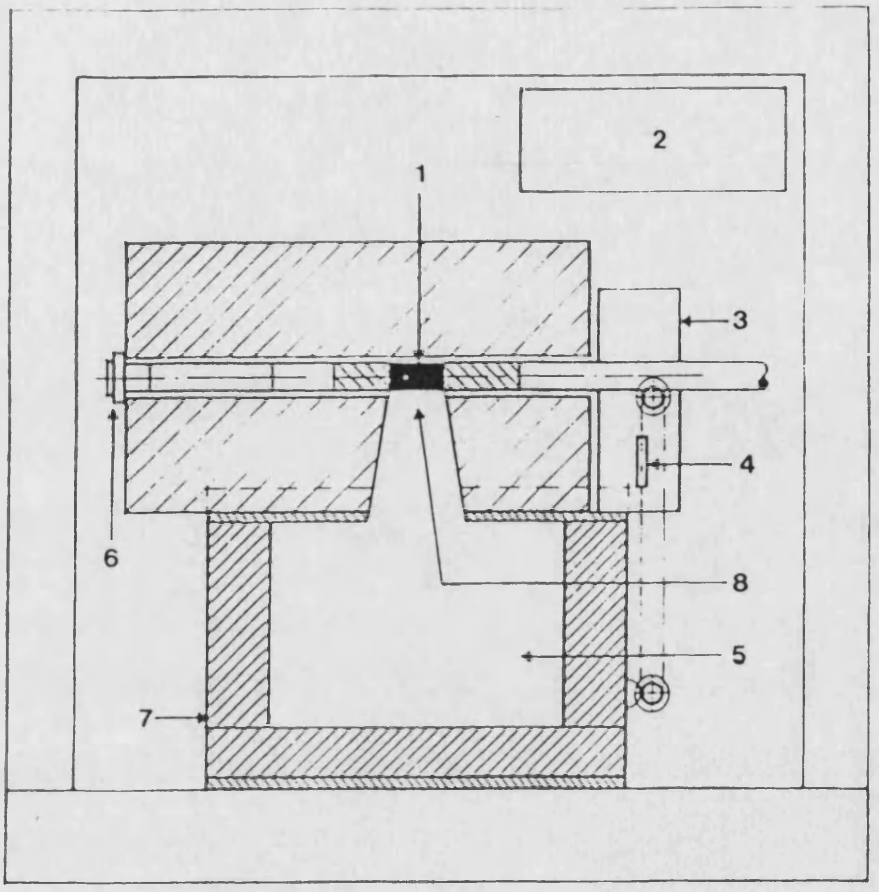


Fig. 2.3.a Vertical Section of Gravatom Caesium-137 Source

1. Caesium-137
2. Control Panel
3. Drive Unit
4. Fail Safe Weight
5. Irradiation Chamber
7. Lead Shield
8. Position of Irradiation Vessel

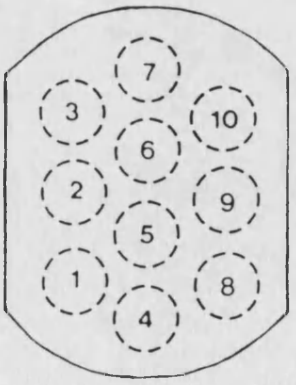


Fig. 2.3.b Plan View of the 10 Vessels Irradiation Jig

GENERAL METHODS

Cleaning glassware: Irradiation vessels and volumetric glassware were immersed in chromic acid for 2 hours, rinsed seven times with tap water and then three times with single distilled water.

Distilled water: The distilled water used in the preparation of all solutions was freshly collected double distilled water.

Gas bubbling in solutions: To avoid any solvent uptake by the bubbled gas, two connected Dreschel bottles stoppered with sintered bottle heads were used. The first bottle contained the appropriate pure solvent used in the experiment, while the second one contained the solution of drug to be bubbled.

Preparation of Alkaline Tetrazolium Blue Reagent[124]

Solution (a) was 0.5% blue tetrazolium in methanol.

Solution (b) was 6N sodium hydroxide in water.

The reagent was prepared by freshly mixing equal parts of solutions (a) and (b).

3. EXPERIMENTAL

3- EXPERIMENTAL

3.1 Dosimetry

The energy imparted to matter by ionising radiation per unit mass of matter is called the absorbed dose, which can be measured by several methods[125]:

1. Calorimetric methods.
2. Ionisation methods.
3. Solid state methods.
4. Chemical methods.

One of the most sensitive methods of dosimetry is the chemical method which is based on the principle that certain quantitative oxidation and reduction reactions are directly proportional to the radiation energy absorbed. The chemical system which has been most extensively investigated is the oxidation of ferrous sulphate in 0.1-0.8N sulphuric acid as first described by Fricke and Morse (1929). This technique is now sufficiently well understood to provide a reliable method of dosimetry[126,127,128,129]. For example, Keene[129] used pulse radiolysis technique to separate and explain some of the radiolytic reactions occurring in the oxidation of ferrous sulphate. With 10mM ferrous sulphate in N sulphuric acid, four stages were observed, as indicated in equations 3.2 - 3.4. The slowest stage (equation 3.4) took several seconds to reach completion and was due to hydrogen peroxide oxidation, the most rapid stage (equation 3.3) was due to hydroxyl radical oxidation to form an uncomplexed

ferric ion. The two intermediate stages (equation 3.2) were consistent with oxidation by hydroperoxy radical to form the hydroxyl complex, followed by a build up of the sulphate complex from the uncomplexed ferric ion.

$$H_2O \rightsquigarrow \dot{H}O_2 + \dot{O}H + H_2 + H_2O_2 \quad \ldots\ldots\ldots \quad 3.1$$

$$Fe^{++} + \dot{H}O_2 + H^+ \longrightarrow Fe^{+++} + H_2O_2 \quad \ldots\ldots\ldots \quad 3.2$$

$$Fe^{++} + \dot{O}H \longrightarrow Fe^{+++} + \bar{O}H \quad \ldots\ldots\ldots\ldots\ldots \quad 3.3$$

$$2\ Fe^{++} + H_2O_2 \longrightarrow 2\ Fe^{+++} + 2\ \bar{O}H \quad \ldots\ldots\ldots \quad 3.4$$

The presence of aliphatic alcohols was found to increase the yield of ferrous ions oxidised on irradiation[130]. The efficiency of an alcohol in bringing about this increased yield was markedly dependent on its structure, and the enhancing action was found to be suppressed by the addition of sodium chloride to the solution. These results were closely parallel to those obtained by Kolthoff and Medalia[131] on the radiolytic oxidation of ferrous ions in aerated aqueous solution containing organic compounds. The enhancing effect of the organic material was supposed to be due to formation of organic peroxide and hydroperoxide by radiolysis, which in turn oxidised the ferrous ions to ferric as follows:

$$RCH_2OH + \dot{O}H \longrightarrow R - \dot{C}HOH \quad \ldots\ldots\ldots\ldots\ldots\ldots 3.5$$

$$\dot{R}CHOH + O_2 \longrightarrow R - \underset{\dot{}}{\overset{O_2}{\overset{|}{C}}}H - OH \quad \ldots\ldots\ldots\ldots \quad 3.6$$

$$Fe^{++} + R - \underset{\dot{}}{\overset{O_2}{\overset{|}{C}}}H - OH + H^+ \longrightarrow Fe^{+++} + R\overset{O_2H}{\overset{|}{C}}HOH \quad .. \quad 3.7$$

$$Fe^{++} + R-\underset{\displaystyle O_2H}{\overset{\displaystyle \cdot}{\underset{|}{CH}}}-OH \longrightarrow Fe^{+++} + R-\overset{\displaystyle \cdot O^-}{\underset{|}{CH}}-OH + OH^- \quad \ldots\ldots \quad 3.8$$

$$Fe^{++} + R - \overset{\displaystyle \cdot O}{\underset{|}{CH}}-OH \longrightarrow Fe^{+++} + RCHO + H_2O \quad \ldots\ldots \quad 3.9$$

and in the presence of a sufficiently high concentration of sodium chloride, the following reaction occurs:

$$Cl^- + \overset{\displaystyle \cdot}{OH} + \overset{\displaystyle +}{H} \longrightarrow \overset{\displaystyle \cdot}{Cl} + H_2O \quad \ldots\ldots\ldots\ldots\ldots \quad 3.10$$

The reaction 3.10 predominates over reaction 3.5. Accordingly, the organic peroxide is not formed leaving the radiolytic products of aqueous solution only to oxidise the ferrous ions to ferric. Therefore, it is necessary to add sufficient concentration of sodium chloride to the dosimetry solution.

The concentration of ferric ions produced can be determined spectrophotometrically at 304 nm[132] and the dose rate calculated from the radiation-induced chemical reaction yield (G-value) of ferric ions from ferrous ions by 100 ev. of deposited energy to 1g of ferrous ammonium sulphate solution.

The dose rate can be calculated as follows:

$$\text{No. of ev./min.} = \frac{\Delta A \times N \times 100}{t \times l \times \epsilon \times G}$$

where:

ΔA = difference between absorbance of irradiated and unirradiated control solution.

N = Avogadro's number (6.022×10^{23}) molecules/mole

l = Optical path length (0.5cm)

ϵ = Molar extinction coefficient of ferric ions in 0.8 N H_2SO_4 at 25°C (2240)[16]

t = time in minutes

G = 15.5 for ferric ions[16]

1 rad = 6.24×10^{13} ev g^{-1}

Therefore:

Number of rad/min =

$$\frac{\Delta A \times 6.022 \times 10^{23} \times 100}{t \times 0.5 \times 2240 \times 15.5 \times 1000 \times 6.24 \times 10^{13}}$$

$$= \frac{\Delta A}{t} \times 55591.25$$

Number of Gray/min = $\frac{\Delta A}{t} \times 555.912$

3.1.1 To Confirm the Characteristics of Cobalt-60 Source by Determining the Dose Rate in a 5 Vessels-Jig

The characteristics of a Cobalt-60 source, as well as its dose rate, are well documented in the department [6,12]. But because of the change of the dose rate by time, it was necessary to carry out some preliminary experimental work to characterise the source through investigation of the dose absorbed by each vessel in the five positions in the jig and to carry out statistical comparison between them.

The dose rate was determined by means of a modified Fricke ferrous ammonium sulphate dosimeter prepared as follows:

1. Stock solutions of 8N sulphuric acid and 10^{-2} M Sodium Chloride were prepared separately by making up 213 ml of sulphuric acid and 0.5850 g of sodium chloride respectively to one litre with distilled water.
2. 25ml of each stock solution were mixed together with 0.0985g of ferrous ammonium sulphate and made up to 250 ml with distilled water.

It was necessary at the beginning to determine the wave-length of maximum absorption of the ferric ions, therefore it was decided to irradiate a single vessel of the dosimetry solution. 2ml sample was irradiated for 45 minutes in a small irradiation vessel using the single vessel jig. Using 0.5cm matched quartz cuvettes, the wave-length of maximum absorption of the irradiated dosimetry solution was determined by scanning manually, on the Unicam SP500 single beam u.v./vis. spectrophotometer, from 250 nm up to 320 nm. against a control

of unirradiated dosimetry solution. From the obtained data, presented in table 3.1.1, and the absorbance spectrum, shown in fig. 3.1.1, the wavelength of maximum absorption was found to be 304 nm.

To determine the dose rate of the Cobalt-60 source and whether the five vessels in the jig received the same dose of radiation, 2ml samples of the dosimetry solution were irradiated in the small irradiation vessels, using the 5 vessels jig, for 5, 10, 15, 20, 25, 30, 45 and 60 minutes. Three readings of the absorbance of each solution against a control of unirradiated dosimetry solution at 304 nm. were noted. The mean absorbances of these three readings were calculated. The experiment was repeated twice, and the mean absorbances of the solution irradiated in each position in the jig, related to time, were submitted to a computerised least squares regression analysis giving the slopes, intercepts, standard deviations and correlation coefficient for the solutions at the five positions (table 3.1.2). Comparing the slopes at the five positions in the jig by applying the t-test, it is evident that the t-values calculated from the slopes are less than the tabulated value (2.36) at 95% confidence limits, as shown in table 3.1.3. This indicates that there is no significant difference between the slopes obtained at the five positions in the jig, and means that the dosimetry solution at each of the five positions in the jig received the same dose of radiation. By calculation, the mean dose rate was found to be 7.484 Gray/minute on 26th October, 1983. The dose rate for the experiments throughout this work were

corrected from that determined on that date for each date of irradiation by using the decay factor for Cobalt as given by Murray[133] and shown in Appendix (I) and occasionally confirmed by irradiation of fresh samples of dosimetry solution.

Table 3.1.1 DETERMINATION OF THE WAVELENGTH OF MAXIMUM ABSORPTION FOR THE DOSIMETRY SOLUTION

Wavelength (nm.)	Mean Absorbance	Wavelength (nm.)	Mean Absorbance
250	0.880	302	0.575
255	0.770	303	0.579
260	0.660	304	0.579
265	0.570	305	0.579
270	0.520	306	0.579
275	0.495	307	0.575
280	0.490	308	0.578
285	0.509	309	0.574
287	0.520	310	0.569
290	0.535	311	0.565
293	0.550	312	0.561
295	0.555	313	0.556
297	0.565	314	0.550
298	0.568	315	0.544
299	0.570	316	0.537
300	0.572	317	0.530
301	0.575	320	0.502

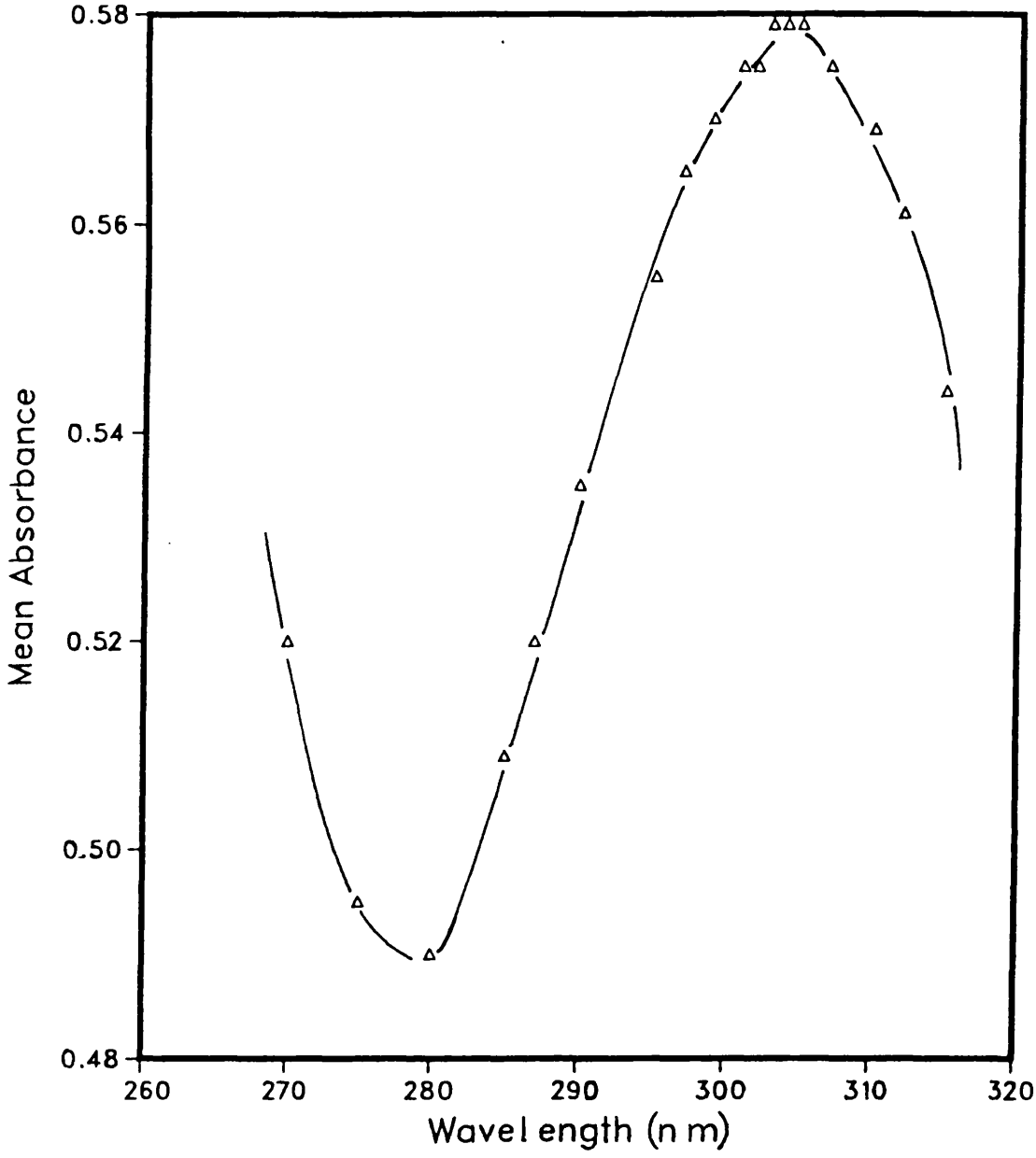


Fig. 3.3.1 Absorbance Spectrum of Irradiated Dosimetry Solution

Table 3.1.2 SLOPE, INTERCEPT, STANDARD DEVIATION AND CORRELATION COEFFICIENT FOR EACH SAMPLE POSITION IN THE 5 VESSEL JIG

POSITION	SLOPE	STANDARD DEVIATION OF SLOPE	INTERCEPT	STANDARD DEVIATION OF INTERCEPT	CORRELATION COEFFICIENT
1	0.01324	0.000188	0.01465	0.005927	0.99939
2	0.01322	0.000184	0.01386	0.005787	0.99941
3	0.01324	0.000188	0.01240	0.005916	0.99939
4	0.01331	0.000173	0.01317	0.005448	0.99949
5	0.01331	0.000181	0.01428	0.005696	0.99944

Table 3.1.3 T-TEST COMPARING THE SLOPES AT THE FIVE POSITIONS IN THE JIG

JIG POSITIONS COMPARED	T-VALUE
1,2	0.215
1,3	0
1,4	0.775
1,5	0.759
2,3	0.215
2,4	1.008
2,5	0.986
3,4	0.775
3,5	0.759
4,5	0

Tabulated t= 2.365 at 95% confidence limits

3.1.2 To Confirm the Characteristics of Caesium-137 Source by Determining the Dose Rate in a 10 Vessels Jig

The use of the 10 vessels' jig (fig.2.3b) had the advantage of allowing irradiation of ten samples simultaneously, but it was necessary to investigate whether the ten vessels received the same dose of radiation especially when the jig was not at the exact centre of the radiation chamber as shown in fig. 2.3a. Therefore, 2ml samples of the freshly prepared ferrous sulphate dosimetry solution were irradiated using the ten vessels jig so that the surface of the solution in each vessel was at a vertical distance of 6 cm under the source and at a position of 30.3 cm from the door and 16.6 cm from the left wall of the radiation chamber. The solutions were irradiated for 5, 10, 20, 25, 30 and 45 minutes. The experiment was repeated twice and the mean absorbance of each solution was calculated (table 3.1.4) and submitted to a computerised least squares regression analysis related to time of radiation. The slope, intercept, standard deviations and correlation coefficient obtained for the solution at each of the ten positions in the jig are presented in table 3.1.5. By calculating the percentage deviation of the dose of radiation absorbed by the solution in each position from the mean absorbed dose (table 3.1.6a), it was found that this percentage deviation was in the range of 0.13-6.27% which was considered to be a high deviation. This means that there was a significant difference in the dose of radiation absorbed by each solution in the ten positions. This could

Table 3.1.4 DATA OF ABSORBANCE FOR EACH DOSIMETRY SOLUTION AT THE TEN POSITIONS IN THE JIG

	ABSORBANCE					
POSITION OF SAMPLE	5 min.	10 min.	20 min.	25 min.	30 min.	45 min.
1	0.074	0.141	0.282	0.338	0.418	0.585
2	0.074	0.139	0.274	0.338	0.413	0.582
3	0.071	0.133	0.260	0.319	0.387	0.552
4	0.075	0.142	0.277	0.346	0.417	0.586
5	0.077	0.143	0.289	0.352	0.427	0.597
6	0.078	0.142	0.278	0.337	0.435	0.592
7	0.073	0.130	0.252	0.310	0.383	0.544
8	0.076	0.142	0.278	0.343	0.420	0.586
9	0.075	0.141	0.282	0.334	0.438	0.594
10	0.078	0.138	0.266	0.331	0.408	0.570

Table 3.1.5 DATA OF SLOPE, INTERCEPT, STANDARD DEVIATION AND CORRELATION COEFFICIENT FOR EACH SAMPLE POSITION IN THE TEN VESSEL JIG

POSITION	SLOPE	STANDARD DEVIATION OF SLOPE	INTERCEPT	STANDARD DEVIATION OF INTERCEPT	CORRELATION COEFFICIENT
1	0.01289	0.000339	0.01616	0.008860	0.99861
2	0.01283	0.000283	0.01446	0.007390	0.99902
3	0.01211	0.000206	0.01450	0.005385	0.99941
4	0.01315	0.000213	0.01308	0.005570	0.99947
5	0.01316	0.000364	0.01797	0.009500	0.99846
6	0.01308	0.000472	0.01604	0.012324	0.99739
7	0.01190	0.000211	0.01406	0.005068	0.99937
8	0.01290	0.000331	0.01706	0.008632	0.99868
9	0.01320	0.000525	0.01344	0.013690	0.99685
10	0.01248	0.000297	0.01749	0.007525	0.99886

Table 3.1.6 COMPARISON OF THE DOSE RATE (GRAY/MIN.) ABSORBED BY EACH OF THE TEN VESSELS THROUGH CALCULATING THE PERCENTAGE DEVIATION FROM THE MEAN

POSITION OF THE VESSEL IN THE JIG	a: POSITION OF THE JIG 30.3cm X 16.6cm IN THE RADIATION CHAMBER		b: POSITION OF THE JIG 29.3cm X 16.4cm IN THE RADIATION CHAMBER	
	DOSE RATE ABSORBED (GRAY/MIN.)	% DEVIATION FROM THE MEAN	DOSE RATE ABSORBED (GRAY/MIN.)	% DEVIATION FROM THE MEAN
1	7.72	0.91	6.15	1.91
2	7.64	0.13	6.32	0.79
3	7.26	5.09	6.23	0.63
4	7.75	1.30	6.22	0.79
5	7.93	3.66	6.39	1.91
6	7.85	2.61	6.36	1.43
7	7.17	6.27	6.31	0.63
8	7.78	1.69	6.22	0.79
9	7.80	1.96	6.33	0.95
10	7.61	0.52	6.21	0.95
	Mean = 7.65		Mean = 6.27	

be because of the eccentric position of the jig in the radiation chamber. To check this the same experiment was repeated after adjusting the position of the jig to be at a distance of 29.3 cm from the door, 16.4 cm from the left wall of the radiation chamber and at a vertical distance of 10 cm from the source. The obtained results are presented in table (3.1.6b) from which the percentage deviation of the dose of radiation absorbed by the solution in each position from the mean absorbed dose was calculated and found to be in the range of 0.63-1.91%. This means that the dosimetry solution in the ten positions in the jig received the same dose of radiation when the jig was exactly at the central position in the radiation chamber. Therefore, it was decided to fix the position of the jig at that central position throughout the work.

Shielding Effect Between the Adjacent Vessels in the 10 Vessels Jig

From the previous experiment, it was evident that each vessel in the jig received the same dose of radiation when all the ten vessels were present in position. But from the design of the Caesium source, the radiation could penetrate each solution perpendicularly through its surface as well as through the wall of the vessel, therefore, the removal of one or more of the surrounding vessels may result in giving more space for radiation for more penetration through the solution. In other words, each vessel may be shielded

from radiation by the surrounding vessels. Therefore, it was decided to investigate this shielding effect by serial removal of some of the vessels from the jig and carrying out dosimetry measurement for the remaining irradiated vessels at a vertical distance of 10 cm from the source and at a position of 29.3 cm from the door and 16.4 cm from the left wall of the radiation chamber. Eight experiments were done. In the first experiment all of the ten vessels containing dosimetry solution were fitted in the jig and irradiated for 25 minutes. In the other seven experiments some of the vessels at different positions in the jig were removed and the remaining vessels were irradiated for 25 minutes. Using 0.5 cm matched quartz cuvettes, three readings of the absorbance of each solution against a control of unirradiated dosimetry solution at 304 nm. were noted. Each experiment was repeated twice, the mean absorbances and accordingly the mean absorbed dose of radiation by each solution were calculated and presented in table 3.1.7.

To compare the mean dose of radiation absorbed by each of the ten vessels in the presence and absence of the surrounding vessels, an F-test was applied from which the calculated F was found to be 9.56 at 95% confidence limits while the tabulated F was 2.18. This means that the dose of radiation absorbed by each vessel was significantly affected by the presence or absence of the surrounding vessels. Throughout the work, it was decided to irradiate the steroid solutions as follows:

Table 3.1.7 THE ABSORBED DOSE RATE (GRAY/MIN.) BY EACH SAMPLE POSITION IN PRESENCE AND ABSENCE OF THE SURROUNDING VESSELS

EXPERIMENT NUMBER	POSITIONS IN THE JIG									
	1	2	3	4	5	6	7	8	9	10
1	6.18	6.33	6.22	6.20	6.40	6.38	6.33	6.20	6.35	6.22
2	X	X	X	6.24	6.42	6.47	6.33	6.29	6.38	6.24
3	6.35	6.49	6.40	X	X	X	X	6.38	6.44	6.27
4	6.18	6.33	6.22	6.27	6.42	6.44	6.33	X	X	X
5	6.18	6.35	6.22	6.31	6.53	6.44	X	6.35	6.40	X
6	6.18	6.33	X	6.33	6.42	X	6.40	6.35	X	6.42
7	6.29	X	6.29	6.38	X	6.51	6.33	X	6.51	6.38
8	X	6.33	6.22	X	6.53	6.53	6.42	6.33	6.42	6.27
Mean	6.22	6.36	6.26	6.28	6.45	6.46	6.35	6.31	6.41	6.30

Grand Mean = 6.34

X = Vacant Position

1. 2 ml samples were irradiated using all the ten vessels simultaneously in the ten vessels jig at a vertical distance of 6cm from Caesium source.
2. The jig was placed centrally in the radiation chamber and exactly under the source.

3.2 Theory and Basic Features of High Pressure Liquid Chromatography System

High Pressure Liquid Chromatography (HPLC) is a technique used to separate the components of a chemical mixture. These components (solutes) are first dissolved in a liquid solvent and then forced to flow through a chromatographic column under high pressure. In this column, the mixture is resolved into its components depending upon the extent of interaction between the solute components and the stationary phase. HPLC differs from gas chromatography (GC) in that:

1. It operates under higher pressure because liquids (mobile phase) are 20-100 times as viscous as gases.
2. The particle diameter (dimensions) in HPLC is around 50-200 times smaller than for GC because the rate of diffusion of liquids are 3,000-30,000 times lower than gases[134].

Generally, HPLC has the advantage over GC in that it can separate compounds of high polarity, high molecular weight, those which suffer from thermal instability and those which have a tendency to ionise in solution. Eluents for HPLC may comprise water, aqueous buffer solutions, aqueous/organic mixtures, organic liquids or mixtures of organic liquids.

Freshly prepared eluents often contain undesirable dissolved air which can be removed by boiling under reflux or by bubbling with an inert gas such as helium. A fine mesh filter may be placed between the reservoir and the pump (a typical filter has a porosity of about 10 μm) to aid clarification of the mobile phase before entering the column. Typical operating pressures range from 75-1500 psi, and an acceptable pump should be capable of delivering eluent against a column back pressure of not less than 4500 psi. The fluid flow rate from the pump must be both smooth and uniform to obtain reproducible results and to avoid a base line noise due to the sensitivity of the detector. Columns vary in dimensions, but usually they are of 100-250 mm in length and 4-8 mm in bore. The column is coupled directly to the injection unit which may be an injection valve or a micro syringe. Ideally, the sample should be placed centrally on the top of the packing material into 5mm. layer of glass beads, glass wool or porous plastic which tops the packing proper in order to prevent disturbance of the packing material. The eluate from the column passes through a minimum length of tubing which must have a bore not large than 0.25mm. A modern HPLC system gives separation in 1-30 min. A diagrammatic representation of a typical HPLC system is shown in fig 3.2.1.

DEFINITIONS

* Retention time or elution time(t_R) is the time between injection and elution of a solute.
* Retention Volume of a solute is the volume of eluent (v_R) passed into the column during the retention time.

$$v_R = t_R f_V$$

where f_v = the volumetric flow rate.

* Phase capacity or column capacity factor (K') is a measure of the degree of retention of a solute, and defined by:

$$K' = \frac{t_R - t_m}{t_m} = \frac{V_R - V_m}{V_m}$$

where t_m = the retention time of the unretained solute.

V_m = volume of eluent in the column.

* The height equivalent to a theoretical plate (H) is the thickness of a traverse slice of column, and its dimensions are those of column length.

$$H = \frac{1}{16}\left(\frac{W_z}{L}\right)^2 = \frac{1}{16}\left(\frac{W_t}{t_R}\right)^2$$

where W_z = peak width at the base in the elution record measured in distance units.

L = length of the column.

W_t = peak width at the base in the elution record measured in time units.

* The number of theoretical plates is given by:

$$N = \frac{L}{H} = 16\ (L/W_z)^2 = 16\ (L/W_t)^2 = 16(L/W_v)^2$$

The higher value of N the better the resolution that can be expected from the column, and for a good column, N will be between 1000-20,000.

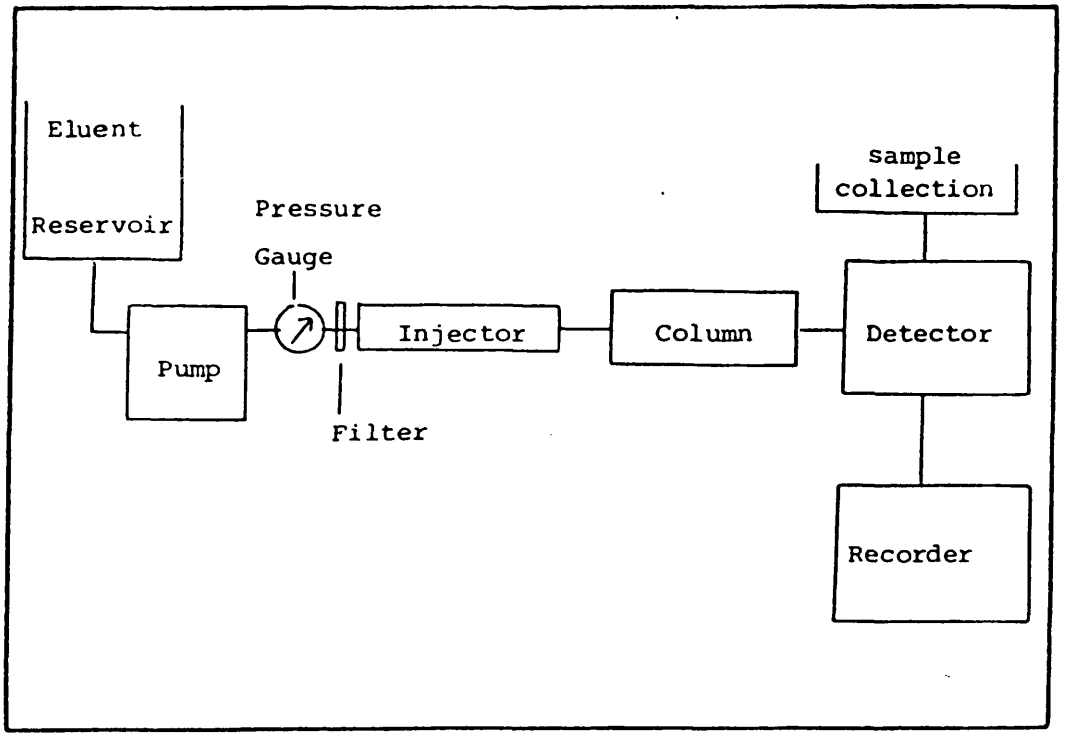


Fig. 3.2.1 Diagrammatic Representation of a Typical HPLC System

3.2.1 Analysis of Corticosteroids

To measure the concentration of corticosteroids quantitatively in the presence of their degradation products, the assay method used must be sensitive, selective and repoducible. The standard B.P.[1,135] assay method uses triphenyl tetrazolium blue as a detection reagent and subsequently determines the absorbance of the coloured complex. However, as triphenyl tetrazolium blue reacts with most reducing steroids with a -$COCH_2OH$- side chain, this method was considered insufficiently selective and a chromatographic method capable of separating the corticosteroid from its degradation products was considered to be the most suitable approach to assaying the drug in the presence of its degradation products. HPLC techniques for corticosteroid separation and analysis are well documented in the literature 65,72,135-145.

Assay of Hydrocortisone and Hydrocortisone Acetate

Several reports have been published in the application of HPLC to hydrocortisone[146-150] and hydrocortisone acetate[151,152] determination in pharmaceutical formulations or biological samples. A standard assay procedure was determined for each drug using a column of 25 cm x 0.46 cm packed with spherisorb ODS of 10 microns particle size as the stationary phase. Preliminary experiments using this column indicated that the best separation for hydrocortisone from its internal standard hydrocortisone acetate was achieved by a mobile phase consisiting of acetonitrile : water (35:65). To separate hydrocortisone acetate from its internal standard deoxycorticosterone a mobile phase consisting of acetonitrile : water

(40:60) gave the best results as shown in fig. 3.2.3, 3.2.4. The flow rate used for both these separations was 1ml/minute and the sample detection was carried out by u.v. absorption at 248nm. which had been determined as the wavelength of maximum absorption for both corticosteroids as presented in table 3.2.1 and shown in fig. 3.2.2.

Using these preliminary findings a calibration curve for a range of concentrations of each of the two corticosteroids was then carried out to assess the reproducibility of such an assay procedure.

Table 3.2.1 DETERMINATION OF WAVELENGTH OF MAXIMUM ABSORPTION FOR HYDROCORTISONE, HYDROCORTISONE ACETATE AND HYDROCORTISONE PHOSPHATE

WAVELENGTH (nm.)	PEAK HEIGHT (cm)		
	HYDROCORTISONE PHOSPHATE	HYDROCORTISONE	HYDROCORTISONE ACETATE
210	4.85	-	-
215	-	4.6	-
220	6.25	5.70	4.94
225	-	7.05	5.77
230	9.32	9.28	7.60
235	12.38	11.98	9.50
239	14.52	14.08	10.85
242	15.98	15.48	11.84
245	16.19	15.95	12.20
248	19.25*	16.25*	12.37*
250	18.72	16.11	11.78
252	-	15.37	11.19
254	17.60	14.50	10.50
256	-	-	9.64
257	-	12.70	-
258	15.25	-	-
260	-	10.42	7.45
265	9.45	7.27	4.89
270	-	3.70	2.55
275	2.42	-	-
280	-	-	-
285	0.80		-

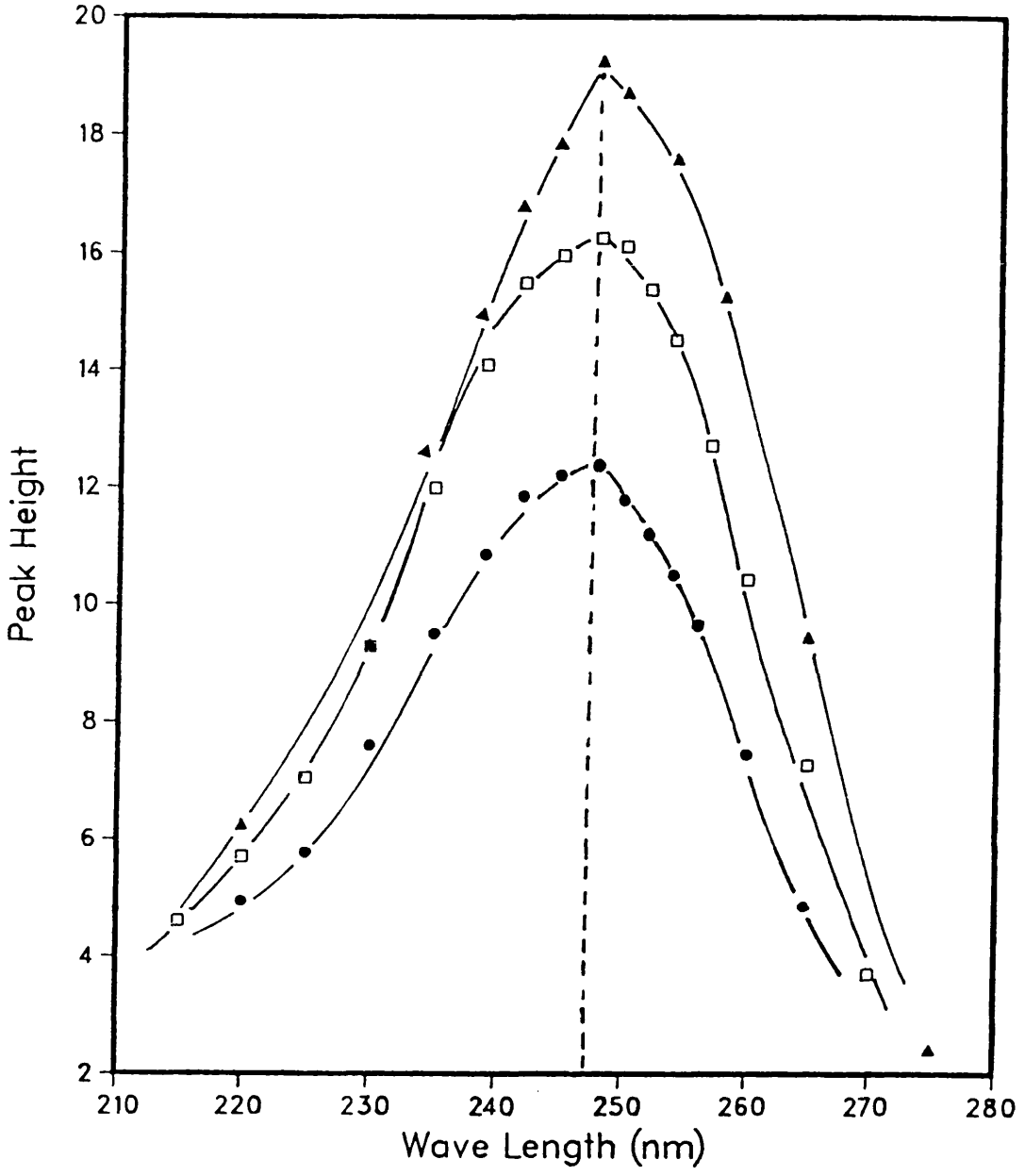


Fig 3.2.2 Absorbance Spectra of Hydrocortisone, Hydrocortisone Acetate and Hydrocortisone Phosphate

▲ Hydrocortisone Phosphate

□ Hydrocortisone

● Hydrocortisone Acetate

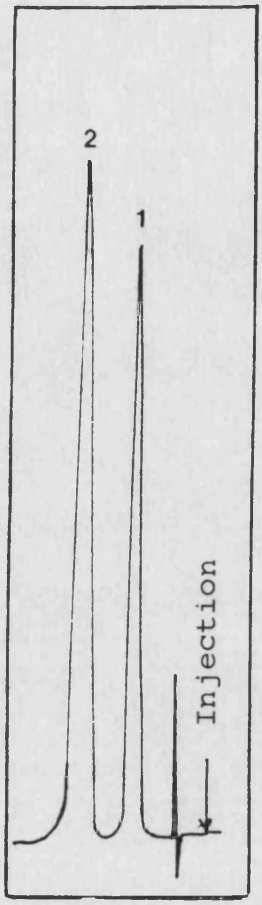


Fig. 3.2.3 Typical HPLC Trace of Hydrocortisone

1. Hydrocortisone (2.068×10^{-4}M)
2. Hydrocortisone Acetate (7.416×10^{-4}M)

Chromatographic Conditions:

Temperature: Ambient Flow Rate: 1.0 ml/min.

Chart Speed: 0.2cm/min.

Absorbance Range: 0.05 AUFS at 248 nm.

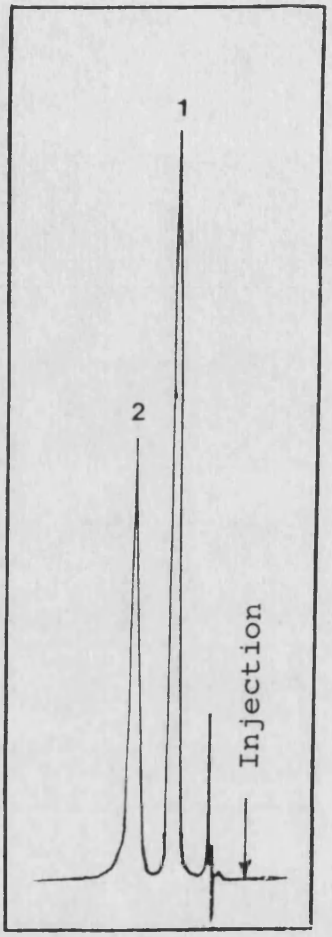


Fig. 3.2.4 Typical HPLC Trace of Hydrocortisone Acetate

1. Hydrocortisone Acetate (6.18×10^{-4}M)
2. Deoxycorticosterone (7.564×10^{-4}M)

Chromatographic Conditions:

Temperature: Ambient Flow Rate: 1.0 ml/min.

Chart Speed: 0.2cm/min.

Asorbance Range: 0.05 AUFS at 248 nm.

Calibration Curves of Hydrocortisone and Hydrocortisone Acetate

The following concentrations of hydrocortisone and hydrocortisone acetate in propylene glycol were prepared:

1. Hydrocortisone:

   0.827×10^{-4}M, 1.034×10^{-4}M, 1.041×10^{-4}M, 1.655×10^{-4}M and 2.068×10^{-4}M

2. Hydrocortisone acetate:

   1.236×10^{-4}M, 2.472×10^{-4}M, 3.708×10^{-4}, 4.944×10^{-4}M, and 6.180×10^{-4}M.

7.416×10^{-4}M and 7.564×10^{-4}M methanolic solutions of hydrocortisone acetate and deoxycorticosterone were also prepared to be used as internal standards for hydrocortisone and hydrocortisone acetate respectively. 1 ml of each concentration, of hydrocortisone or hydrocortisone acetate, was mixed with 1 ml of the respective internal standard in a 10 ml volumetric flask and diluted to 10 ml with methanol. 3 x 20 µl samples of each mixture were injected by means of a loop valve on to the HPLC column and the mean ratio of peak height of the respective corticosteroid to its internal standard, measured at 248 nm., was calculated. The experiment was repeated three times and the four calibrations for each drug were submitted to a computerised least squares regression analysis giving the slopes, intercepts, standard deviations and correlation coefficients all of which are presented in tables 3.2.2 and 3.2.3. A plot of the mean peak height ratios against the concentrations for each drug are shown in figures 3.2.5 and 3.2.6.

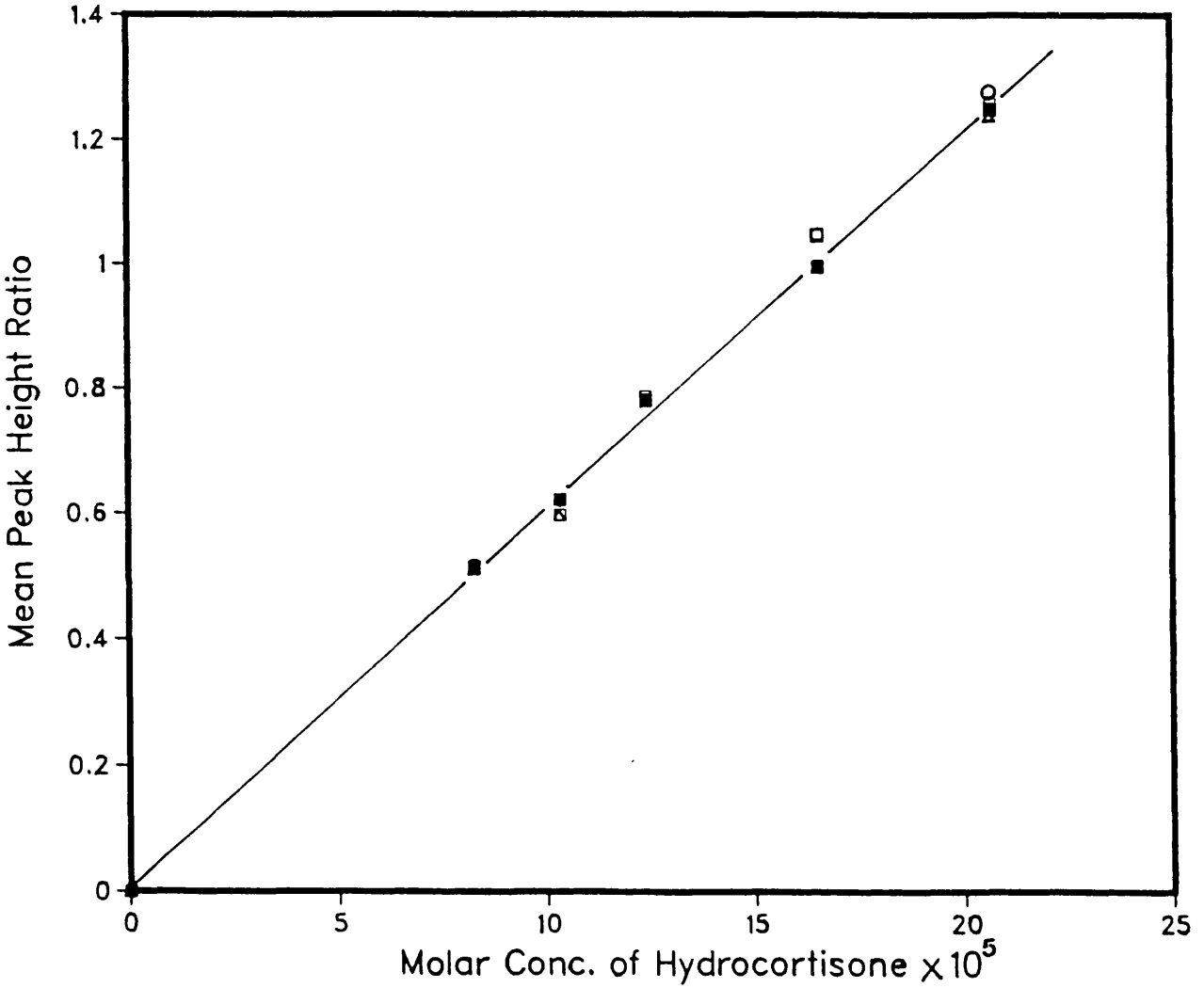


Fig. 3.2.5 Calibration Curve for the HPLC Assay of Hydrocortisone

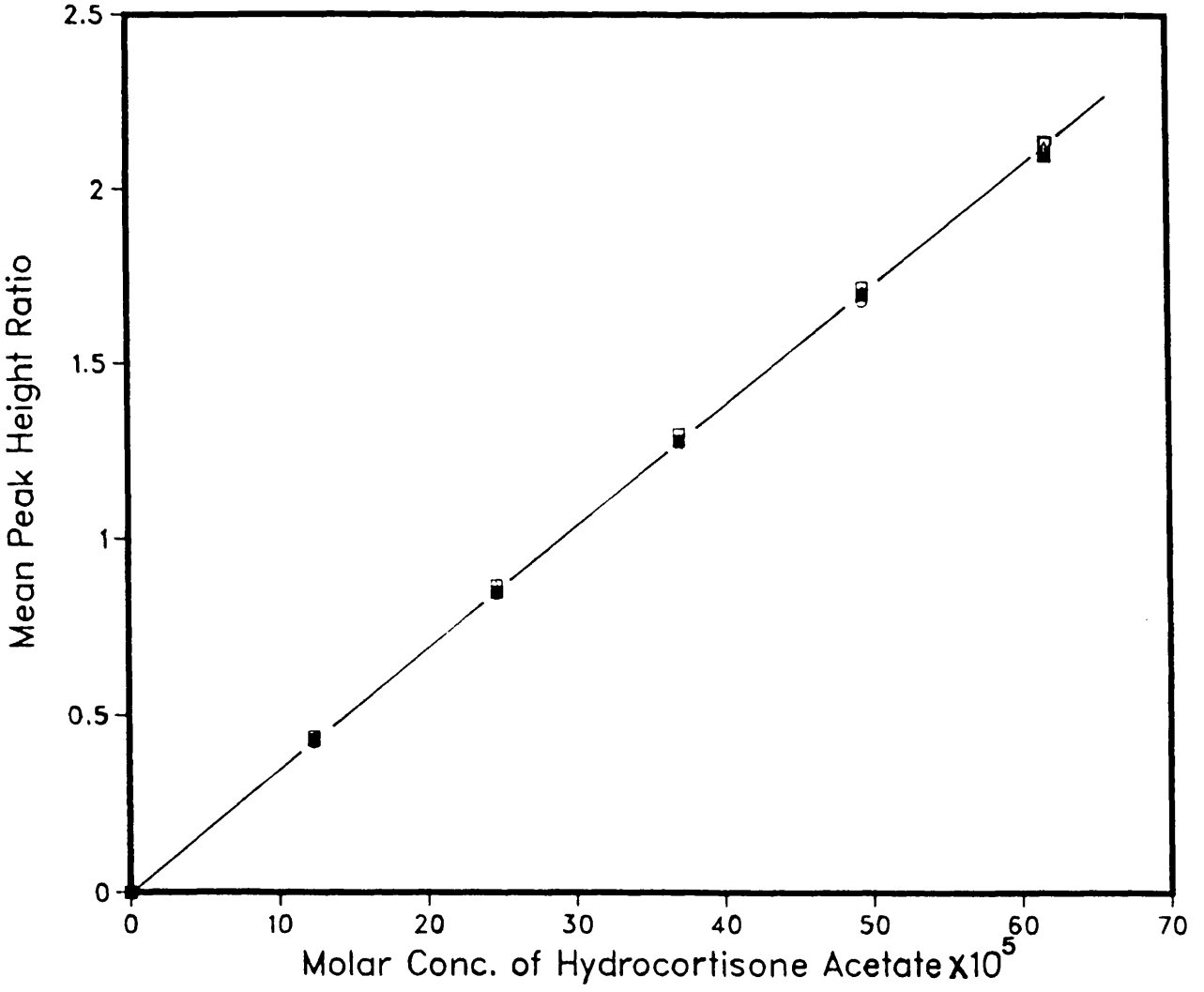


Fig. 3.2.6 Calibration Curve for the HPLC Assay of Hydrocortisone Acetate

Table 3.2.2 DATA FOR REPLICATE CALIBRATION CURVES FOR HPLC ASSAY OF HYDROCORTISONE IN PROPYLENE GLYCOL

CALIBRATION DETERMINATION	SLOPE	STANDARD DEVIATION OF SLOPE	INTERCEPT	STANDARD DEVIATION OF INTERCEPT	t-RATIO ($\frac{\text{SLOPE}}{\text{S.D.}}$)	CORRELATION COEFFICIENT
1	6019.2	254.9	0.00018	0.03660	23.61	0.9974
2	6249.8	303.5	-0.0135	0.04358	20.59	0.9964
3	6193.7	202.9	-0.0032	0.02913	30.53	0.9984
4	5922.9	162.7	0.0247	0.02336	36.41	0.9989

Bartlett Test Slope: Calculated = 1.229 Tabulated = 7.82

P = 0.05 Intercept: Calculated = 0.3197 Tabulated = 7.82

n = 4

Table 3.2.3 DATA FOR REPLICATE CALIBRATION CURVES FOR HPLC ASSAY OF HYDROCORTISONE ACETATE IN PROPYLENE GLYCOL

DETERMINATION	SLOPE	STANDARD DEVIATION OF SLOPE	INTERCEPT	STANDARD DEVIATION OF INTERCEPT	t-RATIO $(\frac{\text{SLOPE}}{\text{S.D.}})$	CORRELATION COEFFICIENT
1	3417.48	17.12	0.0138	0.00702	199.57	1.00
2	3438.51	12.36	0.0190	0.05060	278.23	1.00
3	3407.77	20.81	-0.0024	0.00852	163.79	1.00
4	3383.66	51.33	0.0275	0.02104	65.92	0.9999

Bartlett Test Slope: Calculated = 1.768 Tabulated = 7.82

P = 0.05 Intercept: Calculated = 2.248 Tabulated = 7.82

n = 4

Assay of Hydrocortisone Phosphate

The assay reported by Upton *et al*[153] was chosen for a preliminary investigation of a standard assay procedure for the determination of hydrocortisone phosphate. Using the same column, a mobile phase consisting of methanol : 0.09 M KH_2PO_4 (40 : 60) at a flow rate of 2.3 ml/minute and sample detection by u.v. absorption at 248 nm., the best separation of hydrocortisone phosphate from its internal standard prednisolone 21- sodium succinate was recorded as in fig. 3.2.7.

Calibration Curve of Hydrocortisone Phosphate

2.055×10^{-4}M, 2.877×10^{-4}M, 3.699×10^{-4}M, 4.521×10^{-4}M, and 5.138×10^{-4}M solutions of hydrocortisone phosphate in water were prepared. A 6.217×10^{-4}M solution of prednisolone 21-sodium succinate in water was prepared as an internal standard. 1 ml of each concentration of hydrocortisone phosphate was mixed with 1 ml of the internal standard in 10 ml volumetric flasks and diluted to 10 ml with water. 3 x 20 µl samples of each mixture were injected by means of a loop valve on to the HPLC column and the mean ratio of peak height of hydrocortisone phosphate to the internal standard, measured at 248 nm., was calculated. The experiment was repeated three times and the data were submitted to a computerised least squares regression analysis giving the slopes, intercepts, standard deviations and correlation coefficients, all of which are presented in table 3.2.4. A plot of the mean peak height ratios against the concentration of hydrocortisone phosphate is shown in fig. 3.2.8.

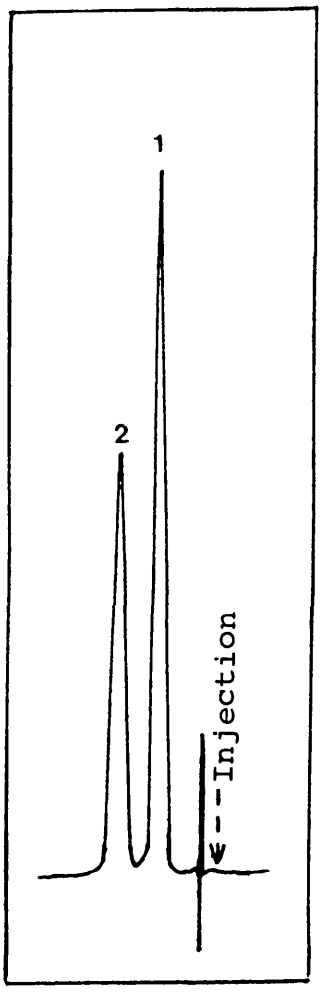


Fig. 3.2.7 Typical HPLC Trace of Hydrocortisone Phosphate

1. Hydrocortisone Phosphate (6.166×10^{-4}M)
2. Prednisolone Sod. Succinate (6.217×10^{-4}M)

Chromatographic Conditions:

Temperature: Ambient Flow Rate: 2.3 ml/min.

Chart Speed: 0.2 cm/min.

Absorbance Range: 0.05 AUFS at 248 nm.

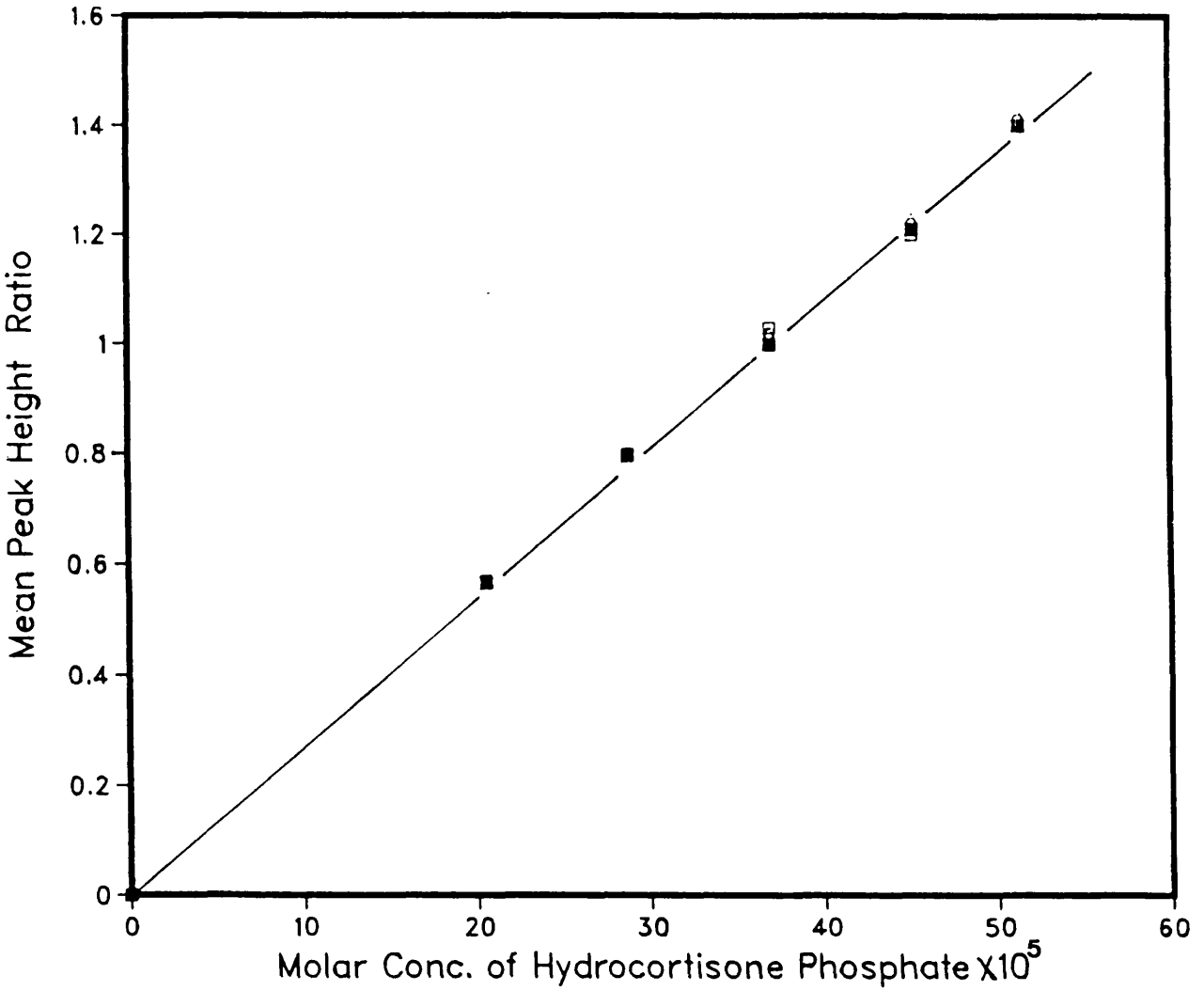


Fig. 3.2.8 Calibration Curve for the HPLC Assay of Hydrocortisone Phosphate

Table 3.2.4 DATA FOR REPLICATE CALIBRATION CURVES FOR HPLC ASSAY OF HYDROCORTISONE PHOSPHATE IN AQUEOUS SOLUTION

DETERMINATION	SLOPE	STANDARD DEVIATION OF SLOPE	INTERCEPT	STANDARD DEVIATION OF INTERCEPT	t-RATIO $(\frac{\text{SLOPE}}{\text{S.D.}})$	CORRELATION COEFFICIENT
1	2758.07	17.75	0.000901	0.00678	155.42	1.00
2	2632.72	85.53	0.04093	0.03260	30.78	0.9984
3	2683.61	47.70	0.02274	0.01823	56.26	0.9999
4	2640.07	68.50	0.02706	0.02618	38.54	0.9989

Bartlett Test Slope: Calculated = 2.720 Tabulated = 7.82

P = 0.05 Intercept: Calculated = 3.077 Tabulated = 7.82

n = 4

From the calibration curves of hydrocortisone, hydrocortisone acetate and hydrocortisone phosphate it is evident that the ratios of slope to the standard deviation of slope were all much greater than 20 and the correlation coefficients are also highly significant at the 95% confidence level indicating good linearity. A Bartlett test (Appendix II) showed no significant difference between either the slope or intercept values. This means that the investigated assay procedures are applicable for the detection and determination of the three steroids. Assuming that any degradation product present, which might absorb at a wavelength of 248 nm., is sufficiently separated or is in such a small concentration that it would neither interfere nor contribute significantly to the peak height, a decrease in the peak height ratio of the parent drug would correspond to a decrease in its concentration. Recognising the limitations imposed by these assumptions, it was decided to proceed with these assay procedures in studying the effect of ionising radiation on the three corticosteroids.

Standard Assay Procedure for Corticosteroids

1 ml of each of the irradiated samples of hydrocortisone, hydrocortisone acetate or hydrocortisone phosphate was pipetted into 10 ml volumetric flasks containing 1 ml of the respective internal standard and the mixtures were then made up to volume with methanol in the case of hydrocortisone or hydrocortisone acetate and with water in the case of hydrocortisone phosphate. 3 x 20 µl samples from each flask were injected by means of a loop valve on to the HPLC column and the mean peak height ratio for each solution was calculated. The experiment was repeated twice and the mean residual concentration of the corticosteroid for each dose of radiation was determined by reference to the unirradiated solution.

3.3.1 Sensitivity of Hydrocortisone, Hydrocortisone Acetate and Hydrocortisone Phosphate to Ionising Radiation

Propylene glycol is well known as a solvent and dispersing agent in the formulation of topical preparations[154], and is recommended as such in the B.P. in the formulation of an ointment[1]. Because of the absence of water, which produces highly reactive radiolytic products, many drugs have been shown to be less sensitive to ionising radiation in non-aqueous topical preparations[5,6,7,8]. However, Hayes[6] has reported the vulnerability of beclomethasone dipropionate to ionising radiation when propylene glycol was present in a non-aqueous ointment. Therefore, it was decided to investigate the sensitivity of hydrocortisone and hydrocortisone acetate in propylene glycol and other organic solvents to ionising radiation and compare it to the sensitivity of hydrocortisone phosphate in water.

The following solutions were prepared:

1. 2.413×10^{-4}M, 3.448×10^{-4}M, 4.482×10^{-4}M, 5.517×10^{-4}M and 6.896×10^{-4}M of hydrocortisone in propylene glycol.
2. 1.840×10^{-4}M, 2.470×10^{-4}M, 3.708×10^{-4}M, 4.980×10^{-4}M and 6.180×10^{-4}M of hydrocortisone acetate in propylene glycol.
3. 2.466×10^{-4}M, 3.452×10^{-4}M, 4.439×10^{-4}, 5.426×10^{-4}M and 6.166×10^{-4}M of hydrocortisone phosphate in water.

2 ml samples of each solution were irradiated in small vessels with different doses of radiation and analysed for the residual concentration of the corticosteroid according to the standard assay procedure. Plots of the residual corticosteroid concentration against dose of radiation are shown in fig. 3.3.1, 3.3.2 and 3.3.3.

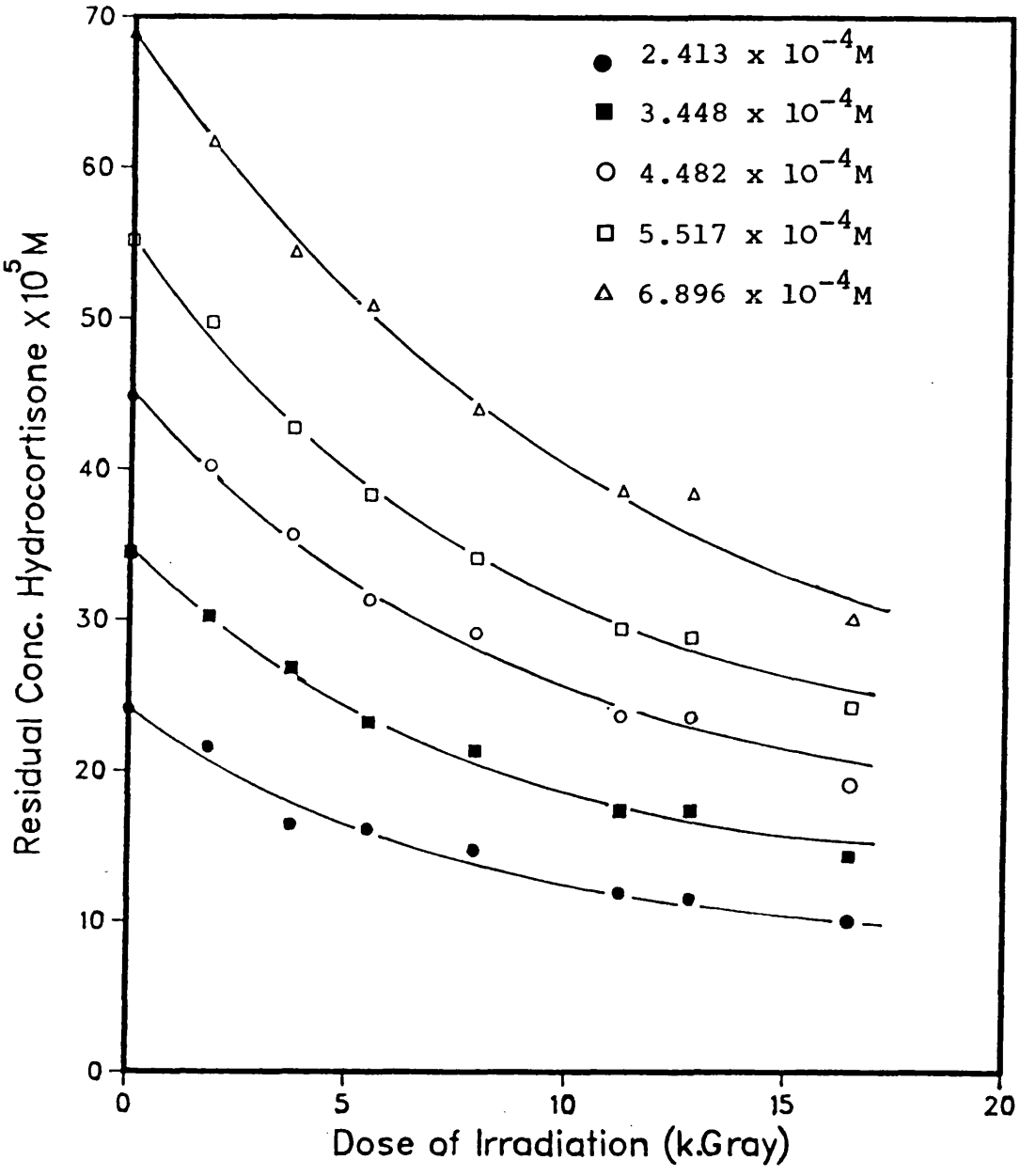


Fig. 3.3.1 The Effect of Initial Concentration on the Sensitivity of Hydrocortisone in Propylene Glycol to Ionising Radiation

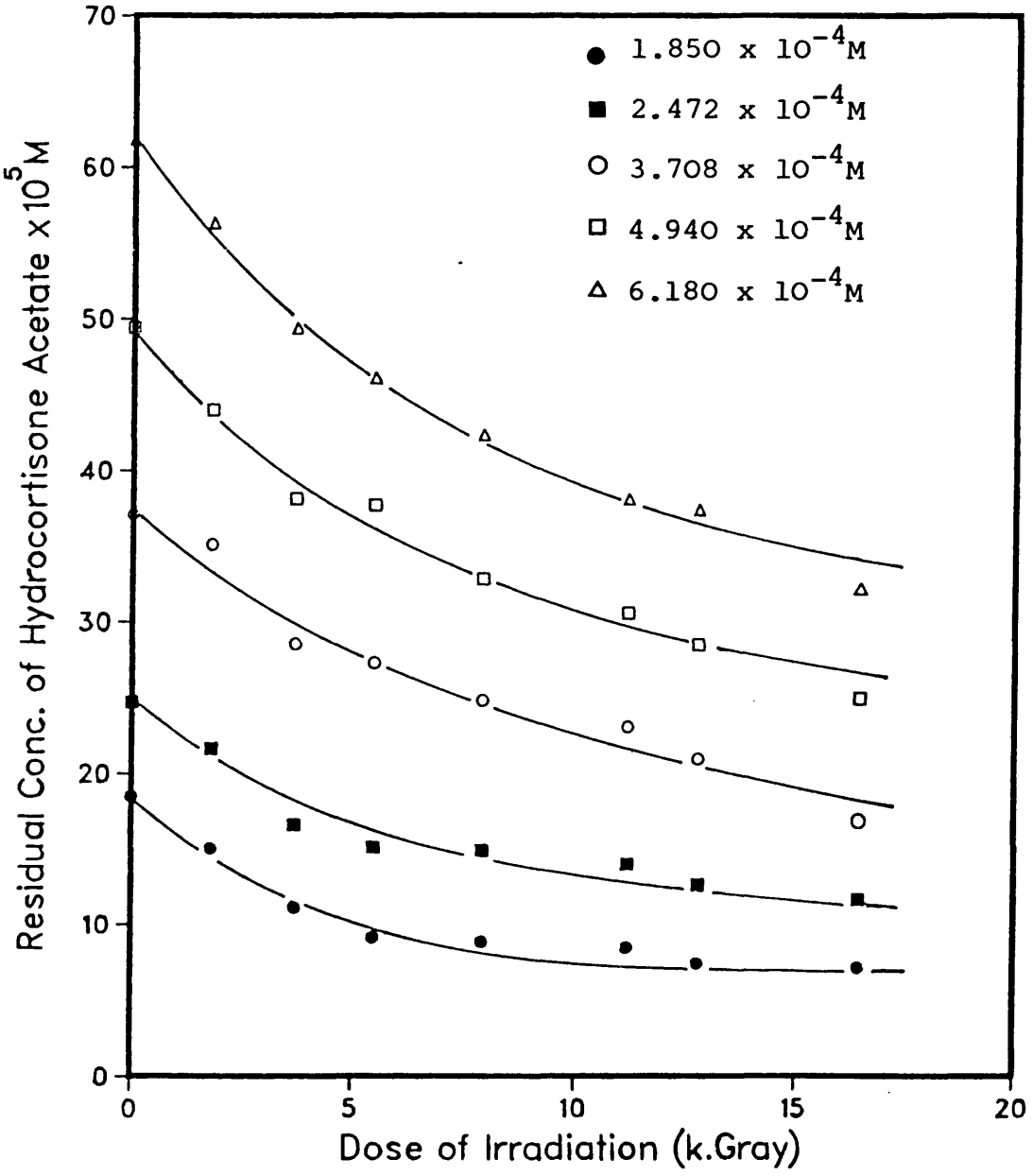


Fig. 3.3.2 The Effect of Initial Concentration on the Sensitivity of Hydrocortisone Acetate in Propylene Glycol to Ionising Radiation

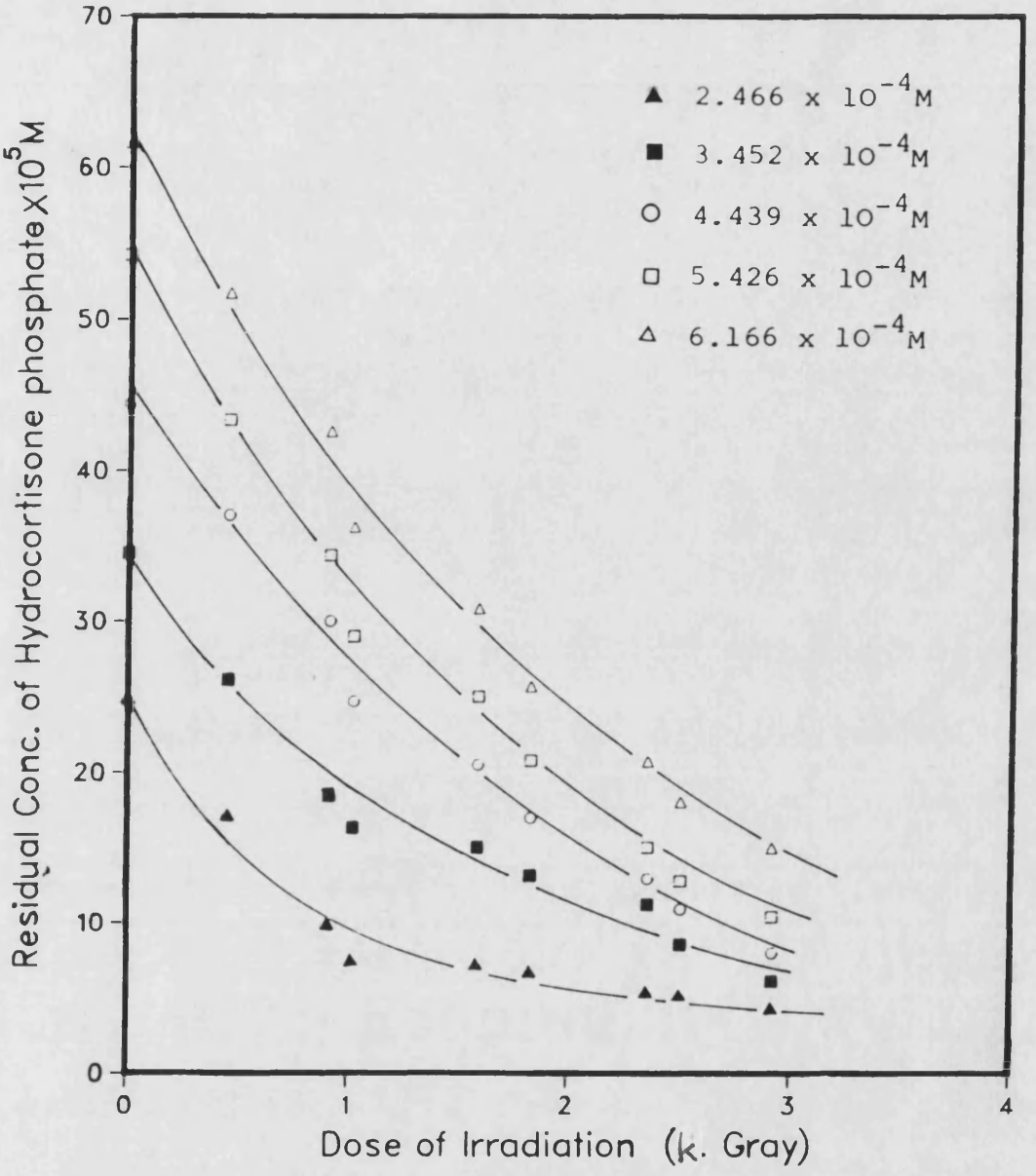


Fig. 3.3.3 The Effect of Initial Concentration on the Sensitivity of Hydrocortisone Phosphate in Water to Ionising Radiation

Treatment of Results

From figs. 3.3.1, 3.3.2 and 3.3.3 it can be seen that the initial slopes of the curves of residual concentrations of hydrocortisone, hydrocortisone acetate and hydrocortisone phosphate against dose of radiation are nearly parallel, indicating that the initial rate of reaction may be constant. This can be confirmed by calculating the order of reaction through the equation:

$$-\frac{dc}{dt} = KC^n$$

where

$\frac{dc}{dt}$ = rate of reaction.

K = reaction rate constant.

c = residual concentration of drug undecomposed.

n = order of reaction.

The equation can be expressed logarithmically as:

$$\log \frac{dc}{dt} = \log K + n \log C$$

The initial rate of reaction can be obtained from the slope of the tangent of the curve at zero dose of radiation and calculated by submitting the greatest number of points to a computerised lease squares regression analysis that gave the maximum value of slope/standard deviation of slope. The apparent initial rates of reaction for each initial concentration of the three corticosteroids in the respective solvents are presented in tables 3.3.1, 3.3.2 and 3.3.3. Plots of log C_o against log initial rate of reaction for the three corticosteroids are shown in fig. 3.3.4, from which the slopes (n) which represent the order of reaction

were found to be 0.235, 0.142 and 0.176 for hydrocortisone, hydrocortisone acetate and hydrocortisone phosphate respectively. Therefore, it is evident that the order of reaction is fractional and highlights the complex nature of the degradation.

Calculation of $G(\pm)$ Value

Reaction yields are usually expressed as the $G(\pm)$ value, which is the number of molecules converted by 100 ev of energy deposited. If the initial rate of reaction is $Y \times 10^{-4}$ mol $l^{-1} K.Gy^{-1}$ of a drug under certain conditions, then 1K.Gy causes change of $Y \times 10^{-4}$ mol l^{-1} of the drug or $Y \times 10^{-4} \times 6.02 \times 10^{23}$ molecules l^{-1} of the drug. As the density of propylene glycol is 1.037 and that of water is unity, the density of the drug solutions in these two solvents is assumed to be unity.

1 K.Gy causes change of $Y \times 10^{-4} \times 6.02 \times 10^{23}$ molecules g^{-1}

As 1 K.Gy of radiation = 6.24×10^{18} ev $gram^{-1}$ of energy

100 ev of energy causes change of

$$\frac{Y \times 10^{-4} \times 6.02 \times 10^{23} \times 10^{-3} \times 100}{6.24 \times 10^{18}}$$

which is G^{-} value of the drug degraded under the specified conditions.

Apparent G^{-} values for each initial concentration of the three corticosteroids in the respective solvents were calculated according to the initial rates of reaction and are presented in tables 3.3.1, 3.3.2 and 3.3.3.

Table 3.3.1 DATA SHOWING LOG C_o, LOG $\frac{dc}{dt}$ AND G^- VALUE FOR EACH INITIAL CONCENTRATION OF HYDROCORTISONE IRRADIATED IN PROPYLENE GLYCOL

INITIAL CONCENTRATION x 10^4M	LOG C_o	INITIAL REACTION RATE $(\frac{dc}{dt})$ x 10^5 mol l^{-1} $K.Gy^{-1}$	LOG $\frac{dc}{dt}$	G^- VALUE
6.896	-3.16	-2.694	4.570	0.259
5.517	-3.25	-2.317	4.636	0.223
4.482	-3.34	-2.049	4.688	0.197
3.448	-3.46	-2.025	4.692	0.196
2.413	-3.61	-2.071	4.684	0.199

Table 3.3.2 DATA SHOWING LOG C_o, LOG $\frac{dc}{dt}$ AND G^- VALUE FOR EACH INITIAL CONCENTRATION OF HYDROCORTISONE ACETATE IRRADIATED IN PROPYLENE GLYCOL

INITIAL CONCENTRATION x 10^4M	LOG C_o	INITIAL REACTION RATE $(\frac{dc}{dt})$ x 10^5 mol l^{-1} $K.Gy^{-1}$	LOG $\frac{dc}{dt}$	G^- VALUE
6.180	-3.20	-2.103	4.677	0.202
4.940	-3.30	-2.019	4.696	0.194
3.708	-3.43	-1.969	4.705	0.189
2.472	-3.60	-1.851	4.732	0.179
1.850	-3.73	-1.751	4.756	0.169

Table 3.3.3 DATA SHOWING LOG C_o, LOG $\frac{dc}{dt}$ AND G^- VALUE FOR EACH INITIAL CONCENTRATION OF HYDROCORTISONE PHOSPHATE IRRADIATED IN WATER

INITIAL CONCENTRATION x 10^4M	LOG C_o	INITIAL REACTION RATE $(\frac{dc}{dt})$ x 10^5 mol l^{-1} $K.Gy^{-1}$	LOG $\frac{dc}{dt}$	G^- VALUE
6.166	-3.20	-20.100	5.696	1.94
5.426	-3.26	-19.021	5.721	1.84
4.439	-3.35	-18.059	5.742	1.74
3.452	-3.46	-17.680	5.752	1.71
2.466	-3.60	-16.914	5.772	1.63

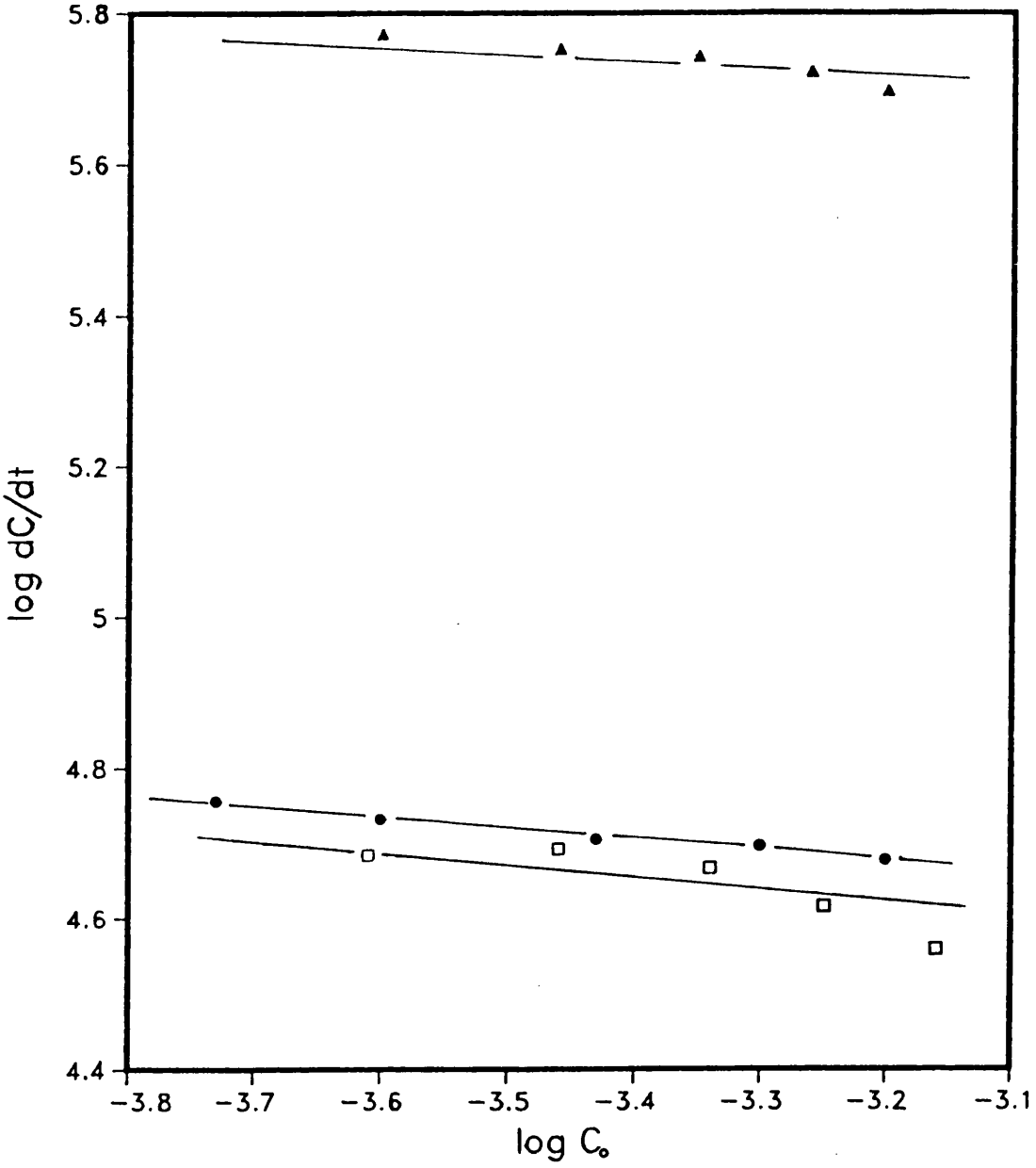


Fig. 3.3.4 Plot of log $\frac{dc}{dt}$ Against log C_o in the Determination of the Order of Reaction for the Three Corticosteroids

▲ Hydrocortisone Phosphate

● Hydrocortisone Acetate

□ Hydrocortisone

3.3.2 The Influence of Different Solvents on the Sensitivity of Hydrocortisone Phosphate to Ionising Radiation

From the results obtained in the previous experiment it was clear that the G^- value of hydrocortisone phosphate in aqueous solution was much higher than that of hydrocortisone and hydrocortisone acetate in propylene glycol. In order to compare the sensitivity of a corticosteroid in both these solvents and in methanol to gamma-radiation, it was decided to study hydrocortisone phosphate's sensitivity because of its high solubility in both water and organic solvents.

6.166×10^{-4}M solutions of hydrocortisone phosphate in water, propylene glycol and methanol were prepared. 2 ml samples of each solution were irradiated in the small vessels with different doses of radiation and analysed by the standard assay procedure for the residual concentrations of hydrocortisone phosphate by reference to the unirradiated solutions as controls. The residual concentrations of hydrocortisone phosphate are plotted against the dose of radiation in fig. 3.3.5 and the reaction rate as well as the G^- value for each solution are presented in table 3.3.4 from which it is evident that the sensitivity of hydrocortisone phosphate to ionising radiation in the three solvents is in the order of water $>$ propylene glycol $>$ methanol. The difference in the sensitivity of hydrocortisone phosphate in methanol and propylene glycol may be due to the different resistance of each of the solvents to γ-radiation and/or the different organic free radicals produced which may react at different

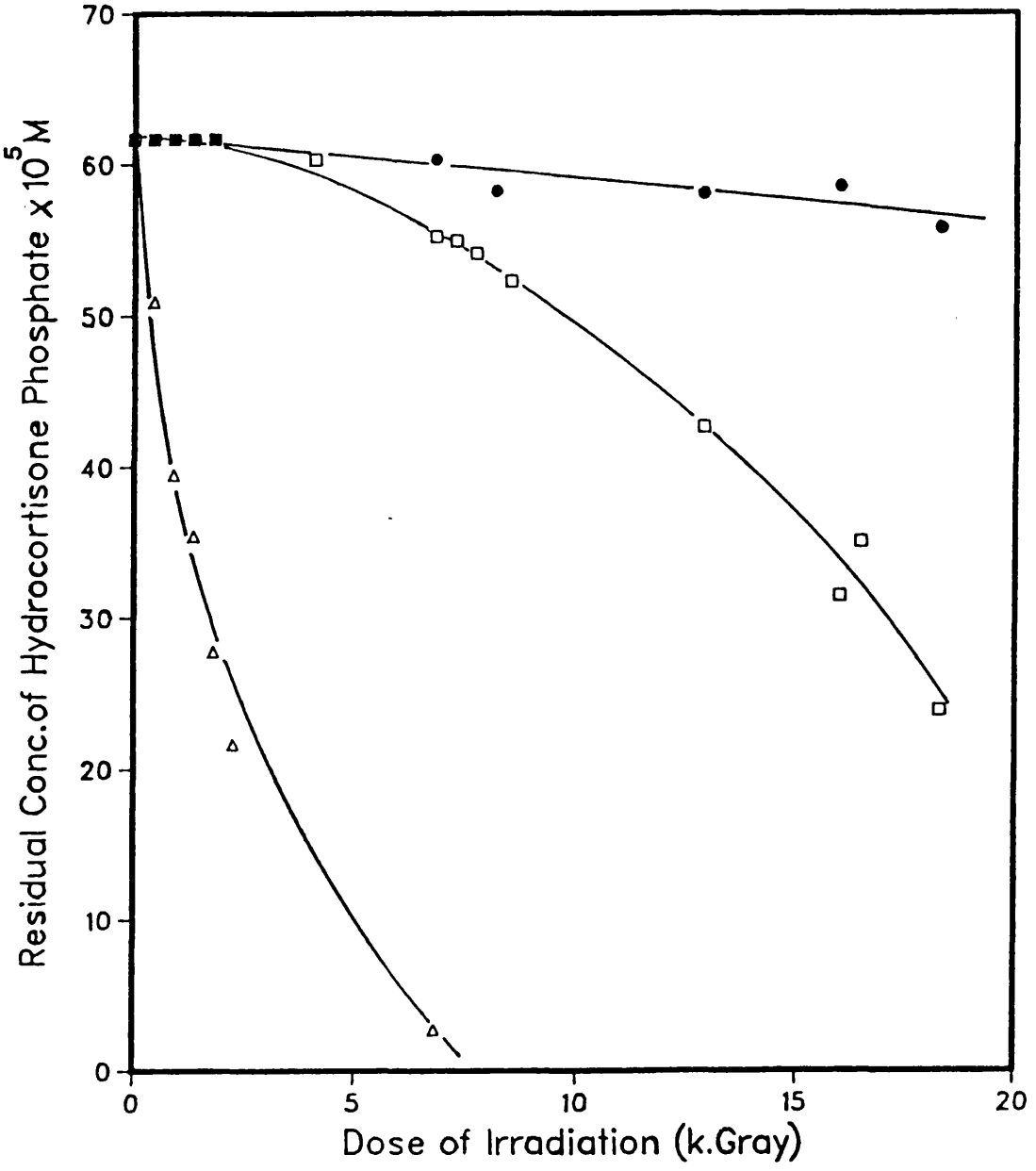


Fig. 3.3.5 The Influence of Different Solvents on the Sensitivity of Hydrocortisone Phosphate to Ionising Radiation

△ Water

□ Propylene Glycol

● Methanol

rates with the corticosteroid. The greater effect observed for water can be assumed to be a result of its highly reactive radiolytic products destroying the steroid.

Table 3.3.4 THE INITIAL REACTION RATES AND G^- VALUES OBTAINED FROM PLOTS OF RESIDUAL CONCENTRATION OF HYDROCORTISONE PHOSPHATE AGAINST DOSE OF RADIATION IN DIFFERENT SOLVENTS.

SOLVENT	INITIAL REACTION RATE x 10^5 mol l^{-1} $K.Gy^{-1}$	G^- VALUE
Water	-19.219	1.85
Propylene Glycol	- 1.094	0.106
Methanol	- 0.292	0.028

3.3.3 Investigation of Effect of the Individual Radicals Produced from Water Radiolysis on Hydrocortisone Phosphate

The significant destructive effect of water on the hydrocortisone phosphate when irradiated compared to that of propylene glycol and methanol is probably due to the highly reactive characteristics of the radiolytic products of water, namely $\dot{O}H$, $\dot{H}$ and solvated electron. This prompted the need to find out which of these three species was primarily responsible for the degradation of the corticosteroid.

A. The Effect of Hydroxyl Radicals

In order to study the effect of hydroxyl radicals on hydrocortisone phosphate, it is necessary to eliminate hydrogen atoms and hydrated electrons from the system. In the presence of nitrous oxide, these two species can be converted into hydroxyl radicals according to the following equations[155]:

$$\bar{e}_{aq}. + N_2O \longrightarrow N_2 + \dot{O}H + \bar{O}H$$

$$\dot{H} + N_2O \longrightarrow N_2 + \dot{O}H$$

Method: To determine the time of bubbling necessary to saturate the solution with nitrous oxide to produce the above reactions, 6.166×10^{-4}M aqueous solution of hydrocortisone phosphate was prepared and divided into three portions:-

Portion I was unbubbled.

Portion II was bubbled with nitrous oxide for half an hour.

Portion III was bubbled with nitrous oxide for one hour.

2 ml samples of each solution were irradiated in the small vessels with different doses of radiation and analysed according to the standard assay procedure for the residual concentrations of hydrocortisone phosphate.

From the results presented in table 3.3.5 it is evident that bubbling N_2O gas for half an hour gave the same results as bubbling for one hour indicating that bubbling N_2O gas for half an hour is quite sufficient to saturate the solution and consequently converts all the hydrated electrons and hydrogen atoms to hydroxyl radicals. A plot of the residual corticosteroid concentration against dose of radiation is shown in fig. 3.3.7 for these two periods of bubbling with N_2O.

B. The Effect of Hydrogen Atoms

To study the effect of hydrogen atoms on hydrocortisone phosphate, the solution requires to be made acidic and then saturated with hydrogen gas to eliminate the hydrated electrons and hydroxyl radicals as follows[155]:

$$\bar{e}_{aq.} + H_3^+O \longrightarrow \dot{H} + H_2O$$

$$\dot{O}H + H_2 \longrightarrow \dot{H} + H_2O$$

Method: Chingpaisal[12] had shown that an aqueous solution at a pH around 1.3 with HCl and bubbled for one hour with hydrogen gas was required to convert all the hydrated electrons and hydroxyl radicals to hydrogen atoms. But according to the results obtained from bubbling N_2O, it was decided that bubbling the aqueous solution with hydrogen gas for half an hour is sufficient for saturation

with the gas.

6.166×10^{-4}M aqueous solution of hydrocortisone phosphate was prepared and the pH was adjusted to 1.27 using 4N HCl. Then the solution was bubbled with hydrogen gas for half an hour. 2ml samples of the solution were irradiated in small vessels with different doses of radiation and analysed according to the standard assay procedure for the residual concentrations of hydrocortisone phosphate. A plot of the residual corticosteroid concentration against dose of radiation is shown in fig. 3.3.7.

C. The Effect of Hydrated Electrons

The effect of hydrated electrons can be studied in an alkaline solution containing methanol, in which the hydroxyl radicals are eliminated and the hydrogen atoms are converted to hydrated electrons according to the following equations[155]:

$$\dot{O}H + CH_3OH \longrightarrow \dot{C}H_2OH + H_2O$$

$$\dot{O}H + \bar{O}H \longrightarrow \bar{O}^{\cdot} + H_2O$$

$$\dot{H} + \bar{O}H \longrightarrow \bar{e}_{aq.} + H_2O$$

Method: It was necessary to carry out a preliminary experiment to determine the appropriate concentration of methanol required to remove all the $\dot{O}H$ radicals. Therefore, 6.166×10^{-4}M hydrocortisone phosphate solutions with pH adjusted at 11 using 1N NaOH[12] were prepared using 0; 10^{-3}M; 10^{-2}M; 10^{-1}M; 1M and 2 Molar methanol as solvents. 2ml samples were irradiated up to 0.91 K.Gy of γ- radiation and then analysed to calculate the peak height ratio of

both irradiated and unirradiated solutions. The experiment was repeated and the mean peak height ratio of both irradiated and unirradiated solutions were plotted against log molar concentration of methanol as shown in fig 3.3.6

The concentration of methanol in the solution which showed a peak height ratio approaching that of the unirradiated solution was found to be 2 Molar which was considered to be the most adequate concentration of methanol for eliminating the hydroxyl radicals from the system. Therefore, an aqueous solution at pH 11 in 2 Molar methanol was used for the investigation of the effect of hydrated electrons on hydrocortisone phosphate. 6.166×10^{-4}M hydrocortisone phosphate solution at pH 11 in 2 Molar methanol was prepared. 2 ml samples were irradiated with different doses of γ-radiation and analysed by the standard assay procedure for the residual concentrations of hydrocortisone phosphate by reference to the unirradiated solution. A plot of the residual corticosteroid concentration against dose of radiation is shown in fig. 3.3.7.

Comparing the effect of the three radiolytic products of water $\dot{O}H$, $\dot{H}$ and hydrated electrons on hydrocortisone phosphate in fig. 3.3.7, it can be observed that only 3.95% of the drug was degraded by the hydrated electron at a dose of 2.29 K.Gy, while approximately 72% of the drug was degraded by $\dot{O}H$ or $\dot{H}$ at the same dose of radiation indicating that the hydrated electron has very little

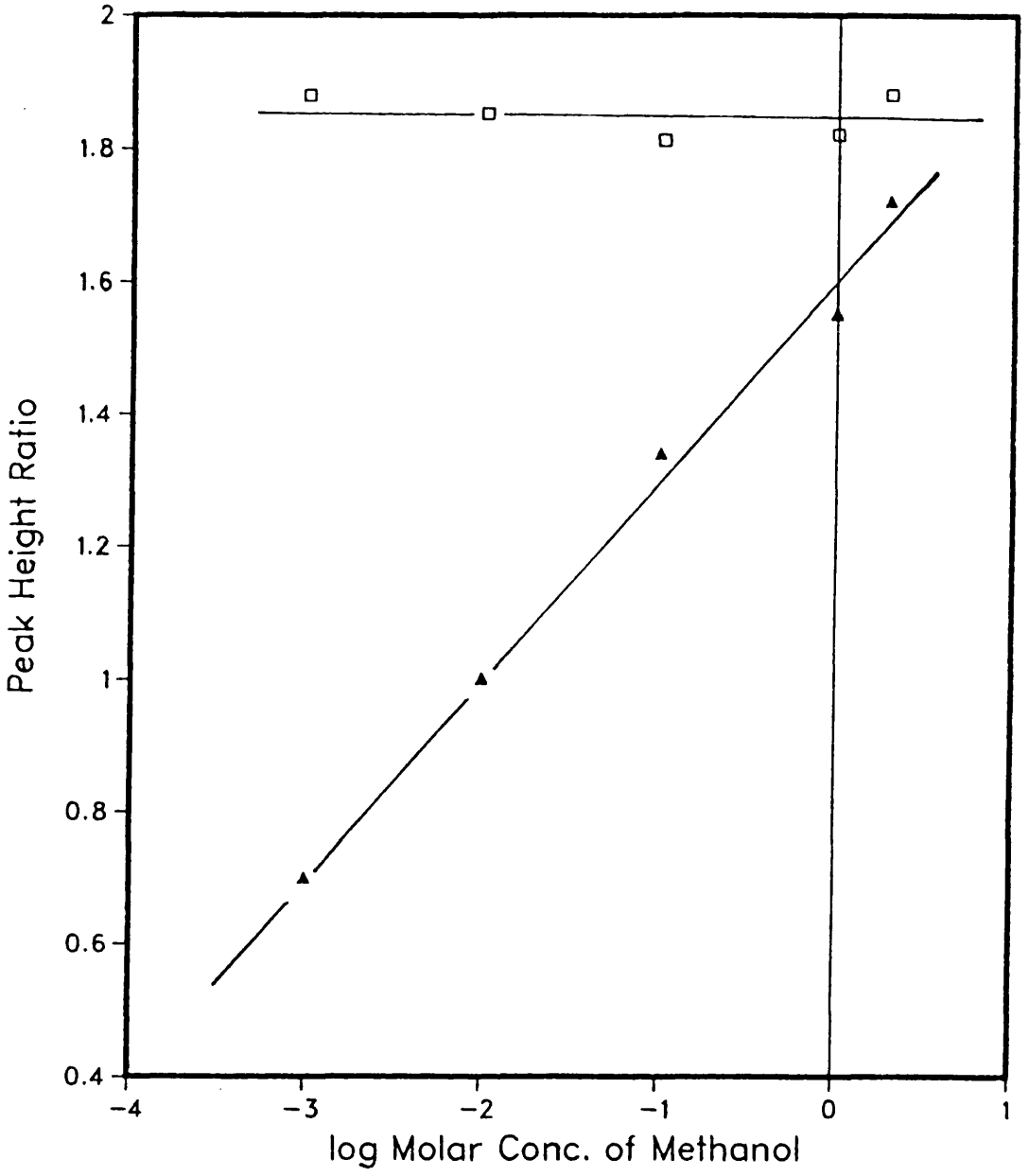


Fig. 3.3.6 Plot of the Peak Height Ratio Against log Molar Conc. of Methanol in Alkaline Aqueous Solution to Determine the Minimum Conc. of Methanol Required to Remove all the Hydroxyl Radicals

□ Unirradiated Solution

▲ Irradiated Solution

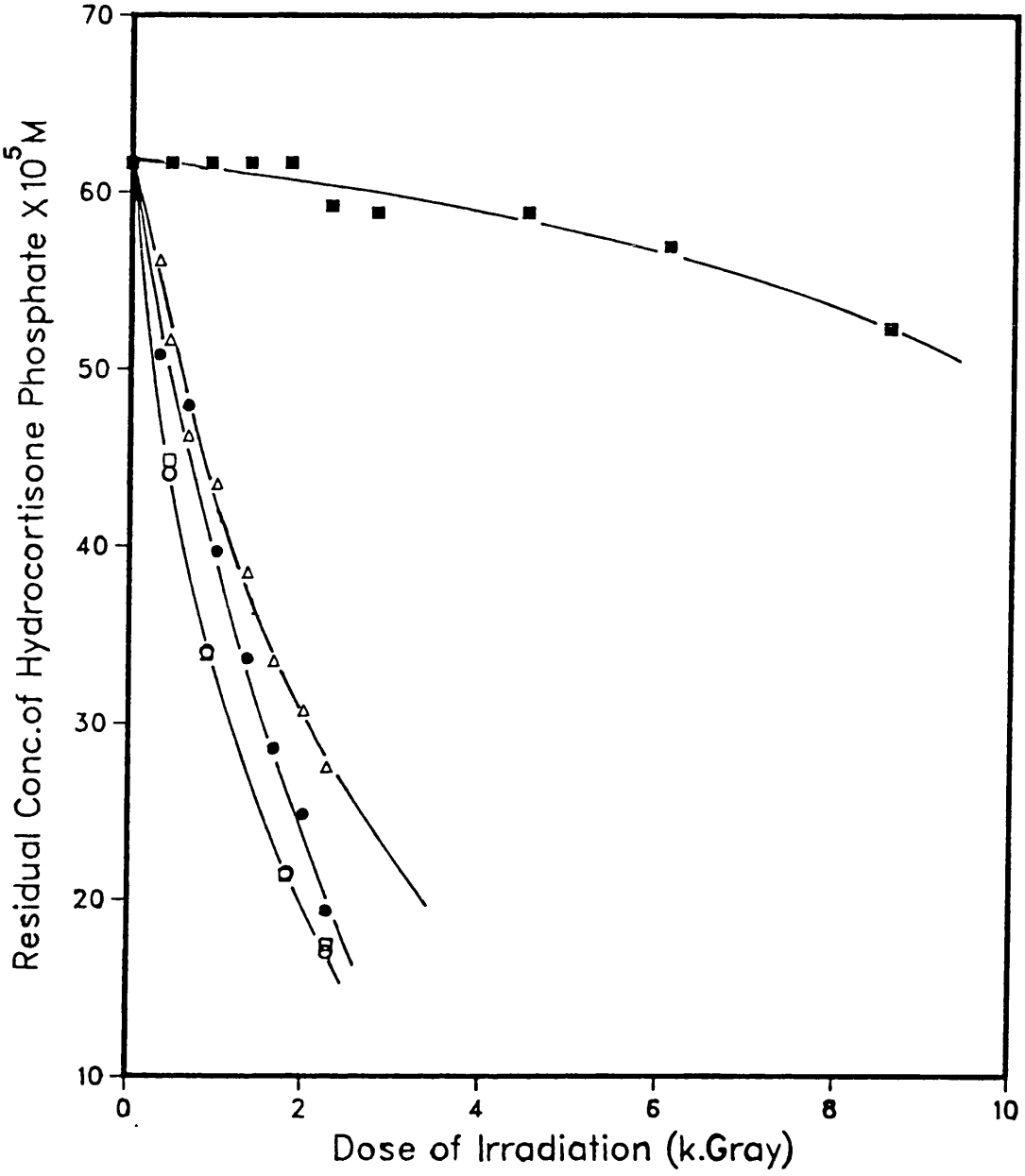


Fig. 3.3.7 The Effect of Hydrogen Atom, Hydroxyl Radical and Hydrated Electron on the Sensitivity of Hydrocortisone Phosphate in Aqueous Solution to Ionising Radiation

△ Water

□ $\dot{O}H$ (by bubbling N_2O for ½ hr.)

○ $\dot{O}H$ (by bubbling N_2O for 1 hr.)

● $\dot{H}$

■ $\bar{e}_{aq.}$

Table 3.3.5 THE EFFECT OF RADIOLYTIC SPECIES OF WATER ON THE G^- VALUE OF HYDROCORTISONE PHOSPHATE

RADIOLYTIC SPECIES	INITIAL REACTION RATE x 10^5 mol l^{-1} k.GY^{-1}	G^- VALUE
Mixture of the radiolytic Products of water ($\dot{O}H + \dot{H} + \bar{e}_{aq.}$)	-19.28	1.86
$\dot{O}H$ (bubbling N_2O for 1 hr)	-30.44	2.94
(bubbling N_2O for ½ hr)	-30.36	2.93
$\dot{H}$	-20.38	1.96
$\bar{e}_{aq.}$	-0.817	0.079

destructive effect on the drug compared to $\dot{OH}$ or $\dot{H}$. However, the effect of the $\dot{OH}$ radicals can be seen to be slightly greater than that of $\dot{H}$ atom as shown by the G^- values presented in table 3.3.5. From the calculated G^- values, the reactivity of the radiolytic products of water is in the order $\dot{OH} > \dot{H} >$ mixture of the three radiolytic products $> \bar{e}_{aq}$.

From all the presented results, it is clear that the aqueous solvent is highly destructive to the corticosteroid when exposed to γ-radiation compared to the organic solvents. However, there is still a significant difference in effect between the organic solvents as shown in the case of methanol and propylene glycol. Therefore it was decided to investigate the influence of chemical structure of the organic solvents on the sensitivity of the corticosteroid to γ-radiation.

3.3.4 The Effect of Different Organic Solvents on the Sensitivity of Hydrocortisone to Ionising Radiation

It has been reported that the number of carbon atoms as well as the extent of branching can greatly affect the sensitivity of aliphatic alcohols to ionising radiation[6,156] which consequently affect the sensitivity of a drug dissolved in these alcohols. Another factor which may affect the sensitivity of aliphatic alcohols to γ-radiation is the number and position of hydroxyl groups in the hydrocarbon chain. To study the influence of this factor, a series of aliphatic alcohols with the same length of hydrocarbon chain and with different numbers and positions of hydroxyl groups were chosen. Because of the higher solubility of hydrocortisone than hydrocortisone phosphate in these solvents, it was decided to investigate its sensitivity in the chosen solvents to γ-radiation.

Method: 6.896×10^{-4}M solutions of hydrocortisone in n-propanol; propylene glycol; 1,3 propanediol and glycerol were prepared separately. 2 ml samples of each solution were irradiated in small irradiation vessels with different doses of radiation and analysed according to the standard assay procedure for the residual concentrations of hydrocortisone by reference to the unirradiated solutions. Plots of the residual concentration of hydrocortisone in the solvents against dose of radiation are shown in fig. 3.3.8.

From the calculated G^- values shown in table 3.3.6 it is evident that the sensitivity of hydrocortisone to

ionising radiation increases in the order n-propanol < propylene glycol < glycerol < 1,3 propanediol. This means that the sensitivity of these aliphatic alcohols increases as the number of hydroxyl groups increases, also the position of these hydroxyl groups in the hydrocarbon chain affects the sensitivity of the alcohol to γ-radiation. This effect of the hydroxyl group's position can be seen from the comparison of propylene glycol and 1,3 propanediol, where the hydroxyl groups are located in 1,2 positions in the hydrocarbon chain in the case of propylene glycol while they are in 1,3 positions in the case of 1,3 propanediol.

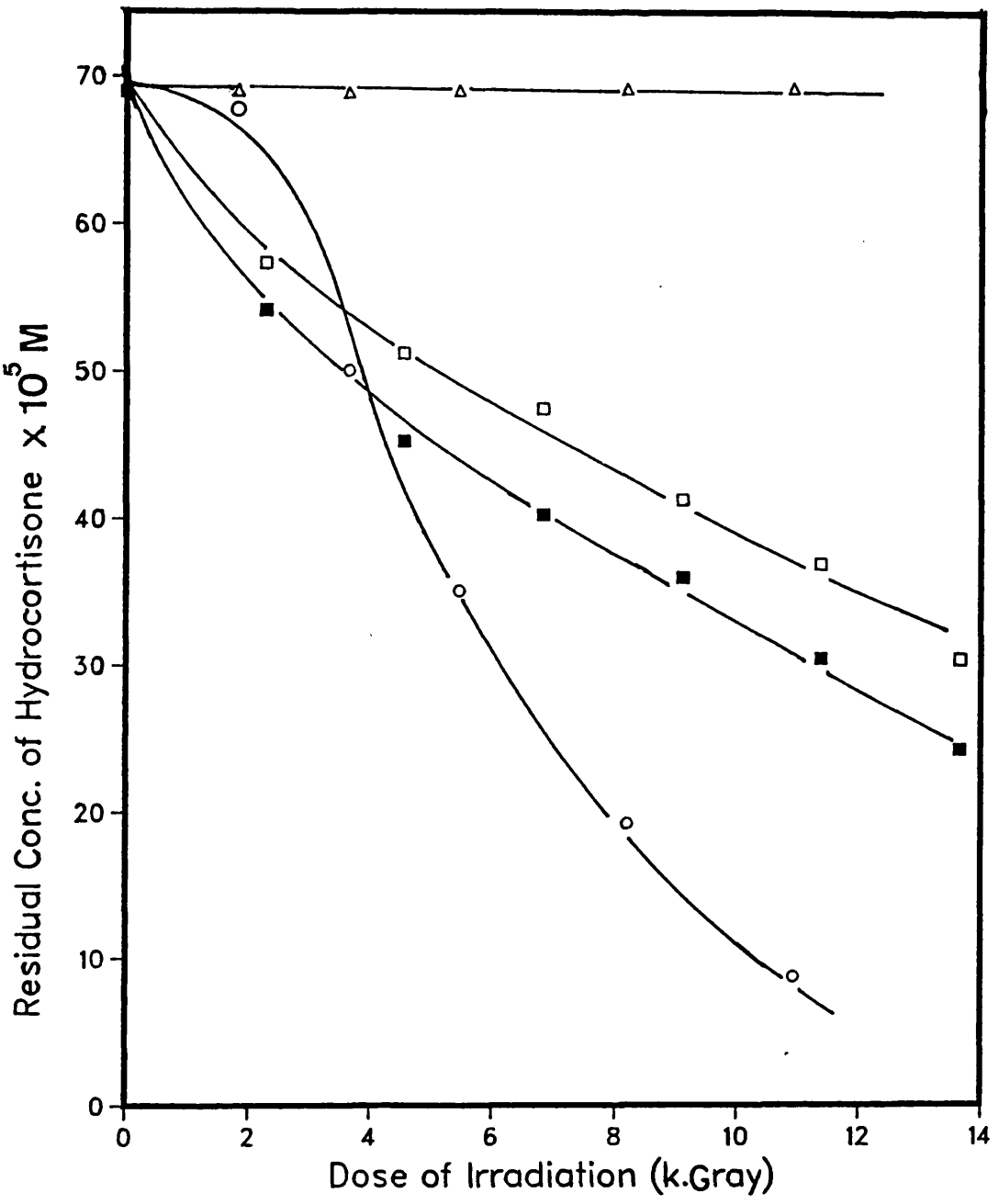


Fig. 3.3.8 The Influence of Different Organic Solvents on the Sensitivity of Hydrocortisone to Ionising Radiation

△ n-Propanol
□ Propylene Glycol
■ Glycerol
○ 1,3 Propanediol

Table 3.3.6 THE SENSITIVITY OF HYDROCORTISONE IN DIFFERENT ORGANIC SOLVENTS TO IONISING RADIATION SHOWN BY REACTION RATE AND G^- VALUE

SOLVENT	INITIAL REACTION RATE x 10^5 mol l^{-1} $k.GY^{-1}$	G^- VALUE
n. propanol	0	0
Propylene Glycol	-2.79	0.269
Glycerol	-3.17	0.306
1,3 propanediol	16.16	0.594

3.4 The Effect of Free Radical Scavengers on the Sensitivity of Hydrocortisone, Hydrocortisone Acetate and Hydrocortisone Phosphate in Different Solvents to Ionising Radiation

The addition of certain substances, in relatively small concentration, to an organic system may markedly affect the radiolytic yield of that system[74]. The mechanism of action of these added substances, called scavengers, may be through their reaction with the primary radiolytic products resulting from radiation. For example, methanol acts as a scavenger for hydroxyl radicals, hydrogen atoms and hydrated electrons resulting from radiolysis of aqueous solutions[27]. Also, molecular iodine is considered to be one of the most effective scavengers in organic systems[76] because of its low activation energy of reaction with radicals together with its relative chemical inertness and that of the resultant iodine radical formed in the reaction:

$$R^{\bullet} + I_2 \longrightarrow RI + I^{\bullet}$$

It was decided therefore to investigate the effect of some scavengers on the sensitivity of hydrocortisone, hydrocortisone acetate and hydrocortisone phosphate in organic and aqueous solutions respectively in order to predict the most destructive radiolytic products on the corticosteroids and compare the relative efficiency of the scavengers under investigation to protect the corticosteroids from ionising radiation.

3.4.1 The Effect of Methanol on the Sensitivity of Hydrocortisone and Hydrocortisone Phosphate in Organic and Aqueous Solvents Respectively to Ionising Radiation

The previous study of the sensitivity of hydrocortisone in different organic solvents showed that the drug was more sensitive in some solvents than others depending on the relative stability of the solvent under investigation to radiation and the reactivity of the respective solvent's radiolytic products with the corticosteroid. The main radiolytic products expected to be produced in the organic solvents are hydrogen atoms, hydroxyl radicals and organic radicals while in aqueous solution, the main species are hydrogen atoms hydroxyl radicals and hydrated electrons. It was decided to study the effect of a free radical scavenger such as methanol on the sensitivity of hydrocortisone and hydrocortisone phosphate in organic and aqueous solutions respectively to ionising radiation.

Method: 6.896×10^{-4}M solutions of hydrocortisone in propylene glycol, glycerol and 1,3 propanediol containing 2.5% v/v; 5% v/v; 10% v/v; 20% v/v and 40% v/v methanol were prepared. Also, aqueous solutions of 6.166×10^{-4}M hydrocortisone phosphate containing 2% v/v and 15% v/v of methanol were prepared. 2 ml samples of each solution were irradiated in small vessels with different doses of radiation and analysed according to the standard assay procedure for the residual concentrations of hydrocortisone and hydrocortisone phosphate by reference to the

unirradiated solutions. The residual concentrations of the corticosteroids are plotted against dose of radiation in fig. 3.4.1, 3.4.2 and 3.4.3.

Plotting the reaction rate of hydrocortisone in propylene glycol, glycerol and 1,3 propanediol, due to radiation, presented in table 3.4.1 against the various percentages of methanol included in the system in fig. 3.4.4 showed that the maximum stability of hydrocortisone in the three solvents can be achieved by the addition of 20% v/v methanol. In the case of aqueous solutions of hydrocortisone phosphate, a significant stabilisation can be noted by the addition of 15% v/v methanol as shown in table 3.4.2 and fig. 3.4.5. This obvious effect of methanol in the aqueous system may be due to its effective scavenging of the radiolytic products of water, mainly hydrogen atoms and hydroxyl radicals.

However, when the experiment was repeated using 2-propanol as an alternative free radical scavenger, which is more reactive to hydrogen atoms and hydroxyl radicals than methanol[27], a more significant stabilisation of hydrocortisone phosphate in the aqueous system was obtained as shown in table 3.4.2 and fig. 3.4.6.

Using mixtures of different proportions of methanol and 2-propanol as scavengers for the radiolytic products of water showed a higher efficiency in stabilising hydrocortisone phosphate than each of the two scavengers individually, as shown in table 3.4.3 and fig. 3.4.7.

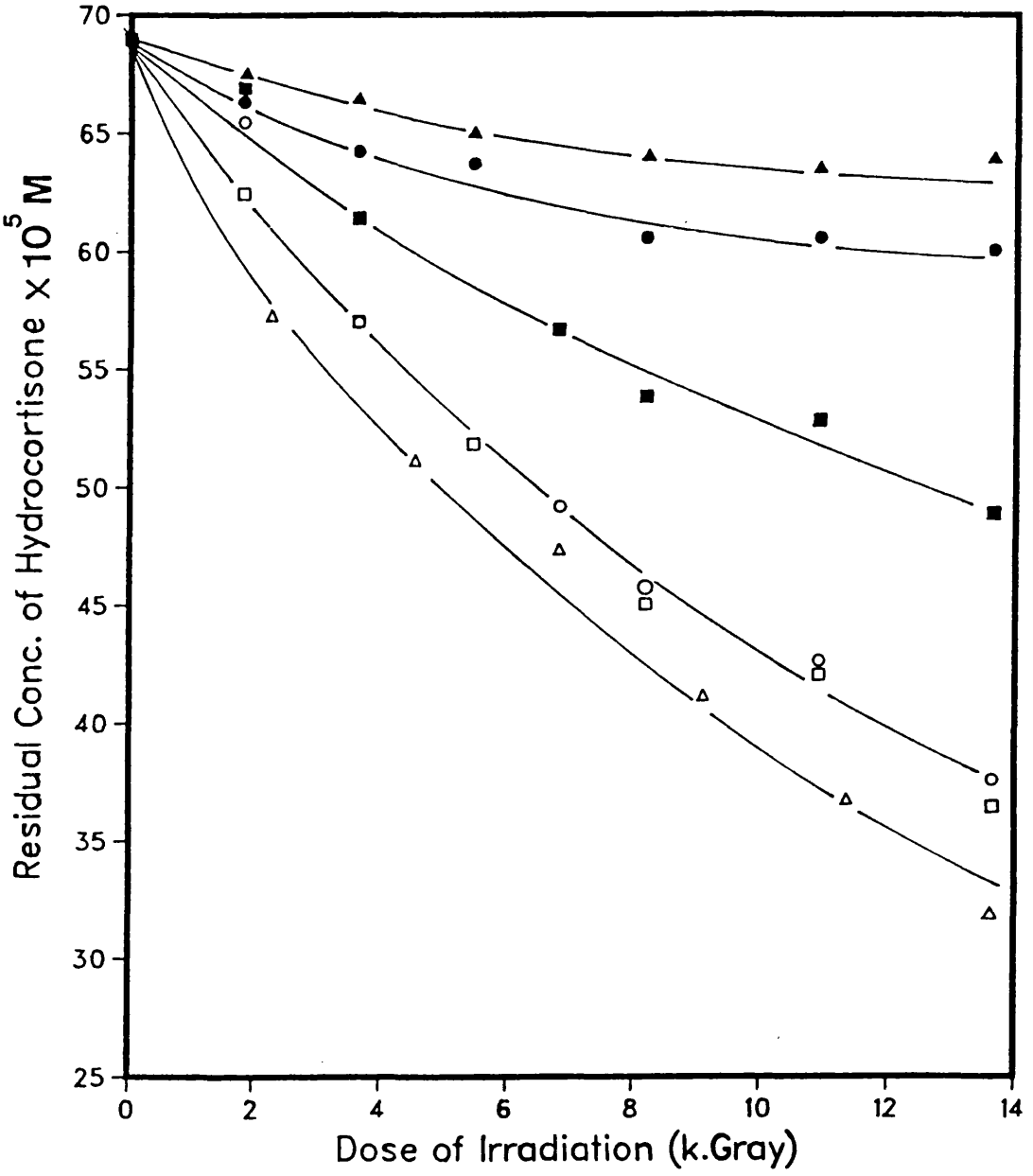


Fig. 3.4.1 The Effect of Methanol Content on the Sensitivity of Hydrocortisone in Propylene Glycol to Ionising Radiation

△ Propylene Glycol
□ 2.5% Methanol
○ 5% Methanol
■ 10% Methanol
● 20% Methanol
▲ 40% Methanol

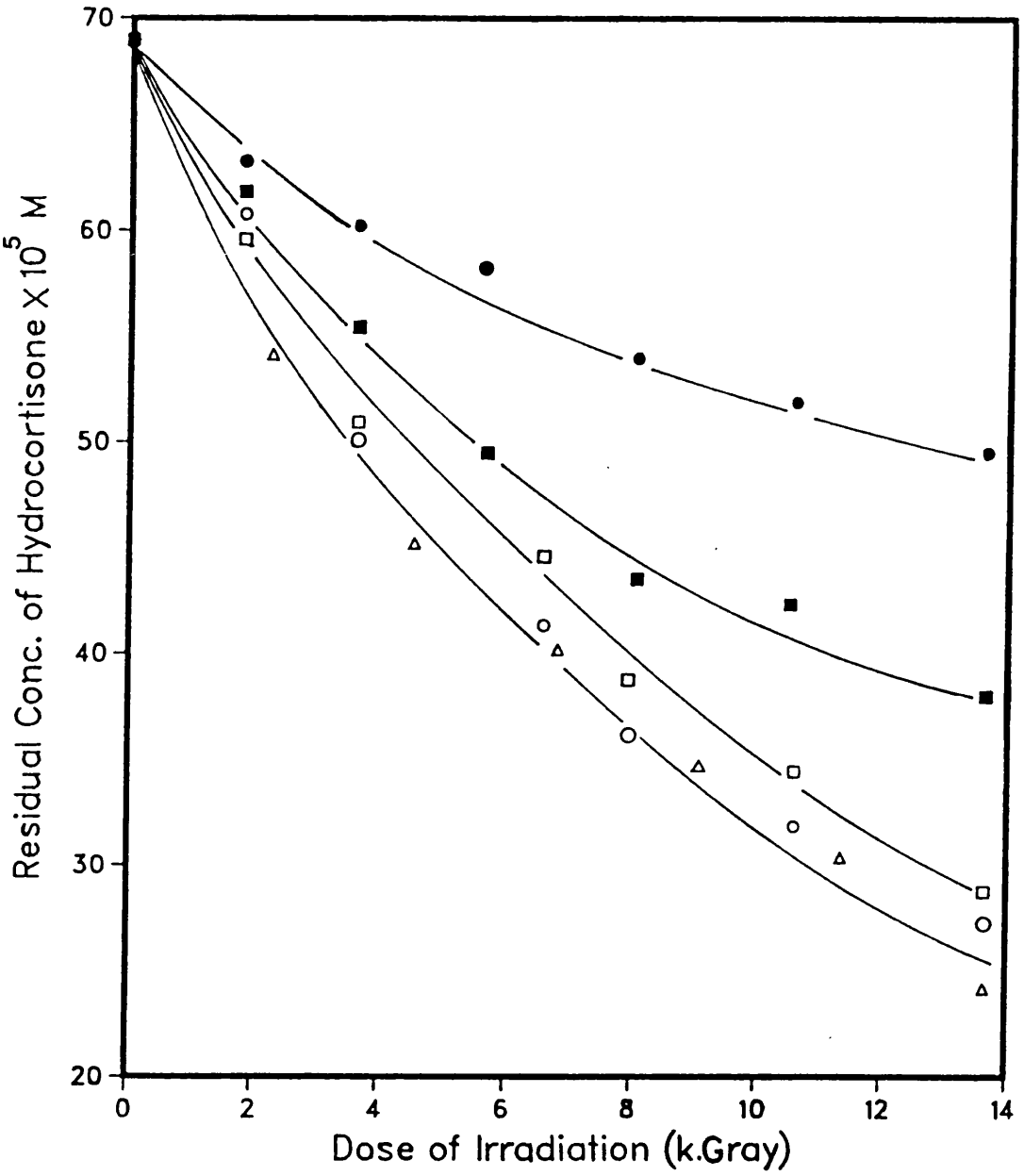


Fig. 3.4.2 The Effect of Methanol Content on the Sensitivity of Hydrocortisone in Glycerol to Ionising Radiation

△ Glycerol
○ 5% Methanol
□ 10% Methanol
■ 20% Methanol
● 40% Methanol

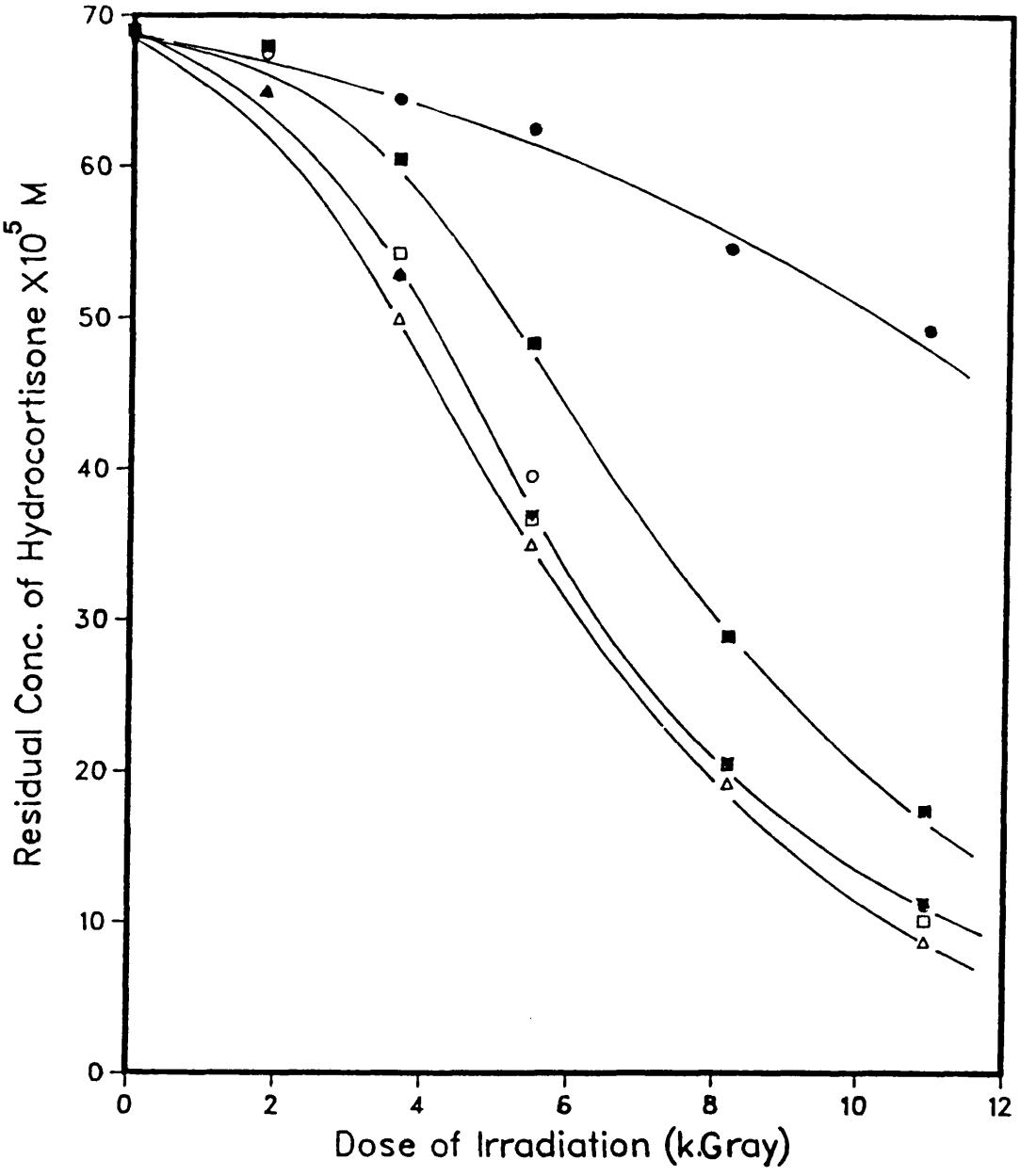


Fig. 3.4.3 The Effect of Methanol Content on the Sensitivity of Hydrocortisone in 1,3 Propanediol to Ionising Radiation

△ 1,3 Propanediol
□ 2.5% Methanol
○ 5% Methanol
▲ 10% Methanol
■ 20% Methanol
● 40% Methanol

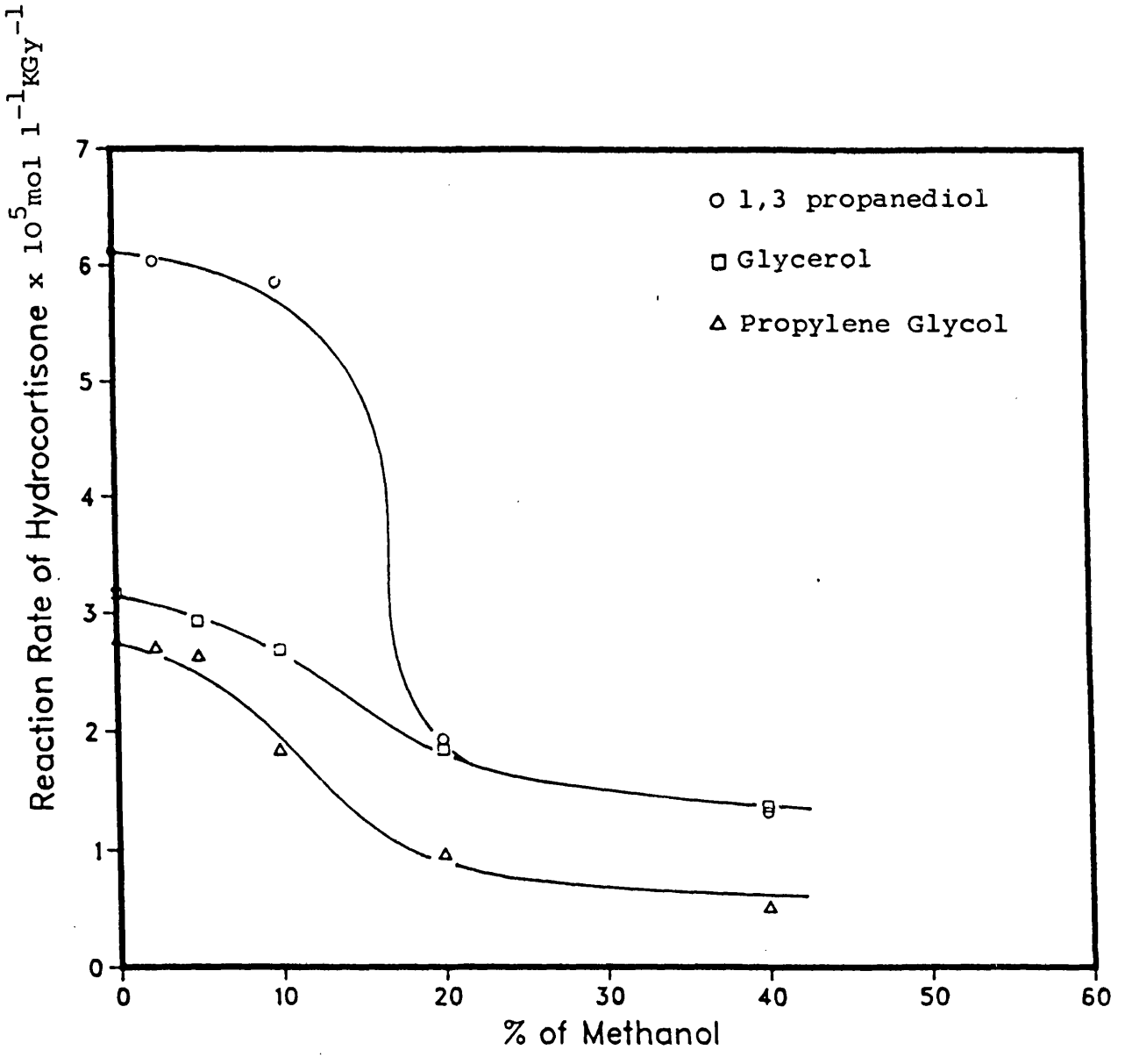


Fig. 3.4.4 The Effect of Methanol Content on the Sensitivity of Hydrocortisone in Organic Solvents to Ionising Radiation

Table 3.4.1 THE EFFECT OF METHANOL ON THE SENSITIVITY OF HYDROCORTISONE IN DIFFERENT ORGANIC SOLVENTS TO IONISING RADIATION

THE PERCENTAGE OF METHANOL ADDED TO SOLUTION (v/v)	INITIAL REACTION RATE x 10^5 mol l^{-1} $K.Gy^{-1}$		
	PROPYLENE GLYCOL	GLYCEROL	1,3 PROPANEDIOL
0	-2.78	-3.17	-6.12
2.5	-2.71	-	-6.04
5	-2.64	-2.93	-5.86
10	-1.85	-2.69	-5.08
20	-0.973	-1.85	-1.94
40	-0.518	-1.37	-1.32

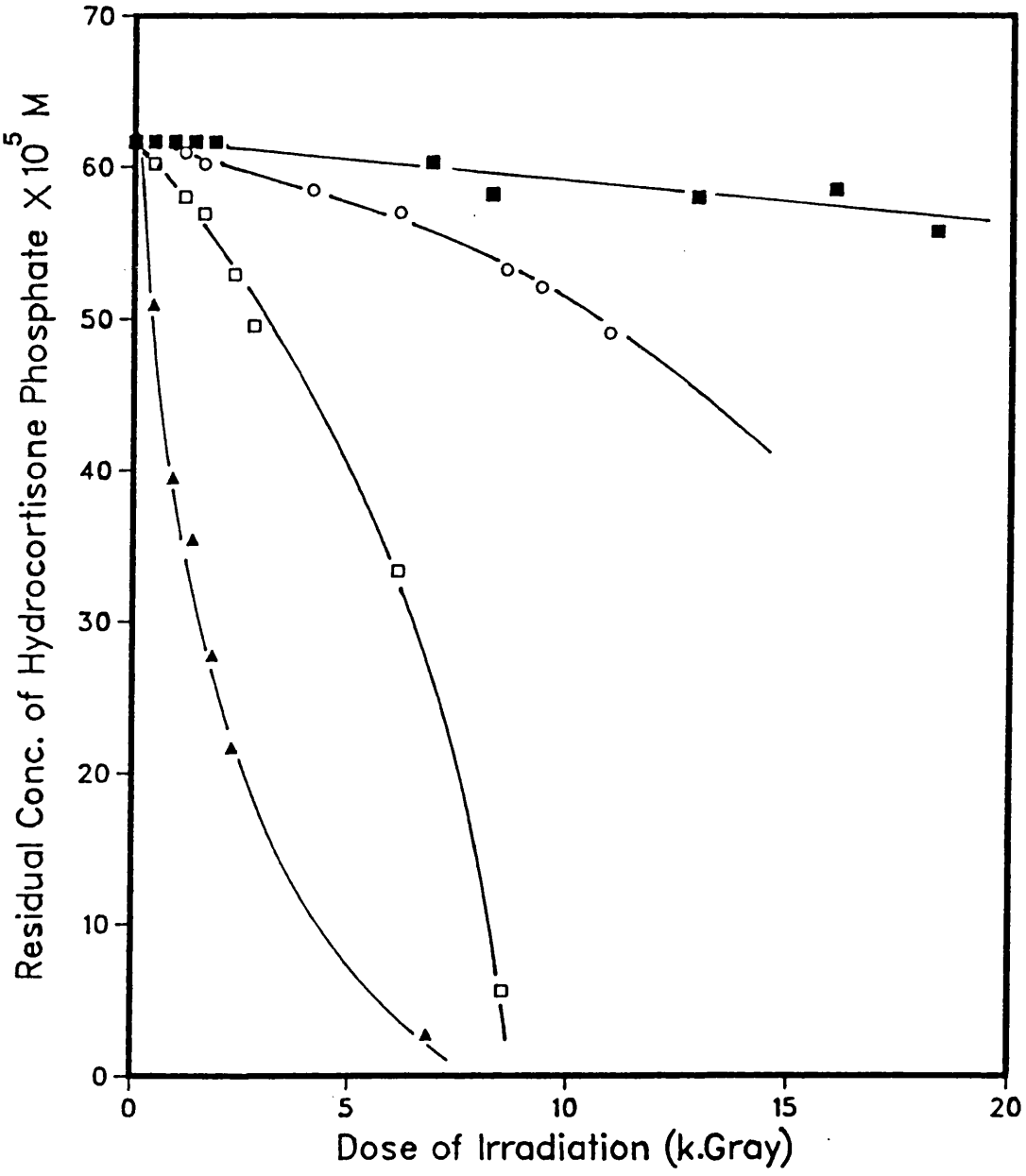


Fig. 3.4.5 The Effect of Methanol on the Sensitivity of Hydrocortisone Phosphate in Water to Ionising Radiation

▲ Water

□ 2% Methanol

○ 15% Methanol

■ 100% Methanol

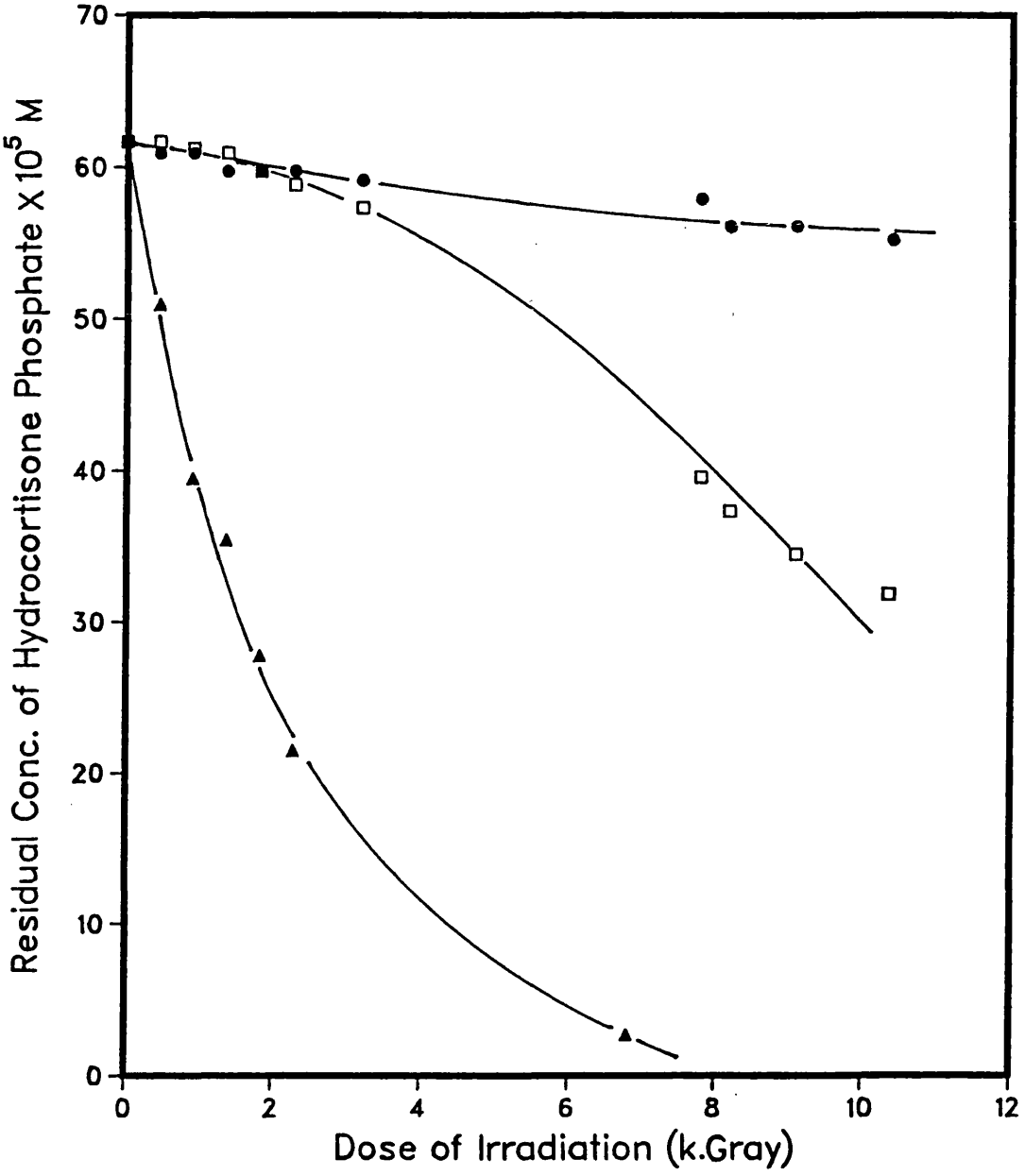


Fig. 3.4.6 The Effect of 2-propanol on the Sensitivity of Hydrocortisone Phosphate in Water to Ionising Radiation

▲ Water

□ 2% 2-propanol

● 15% 2-propanol

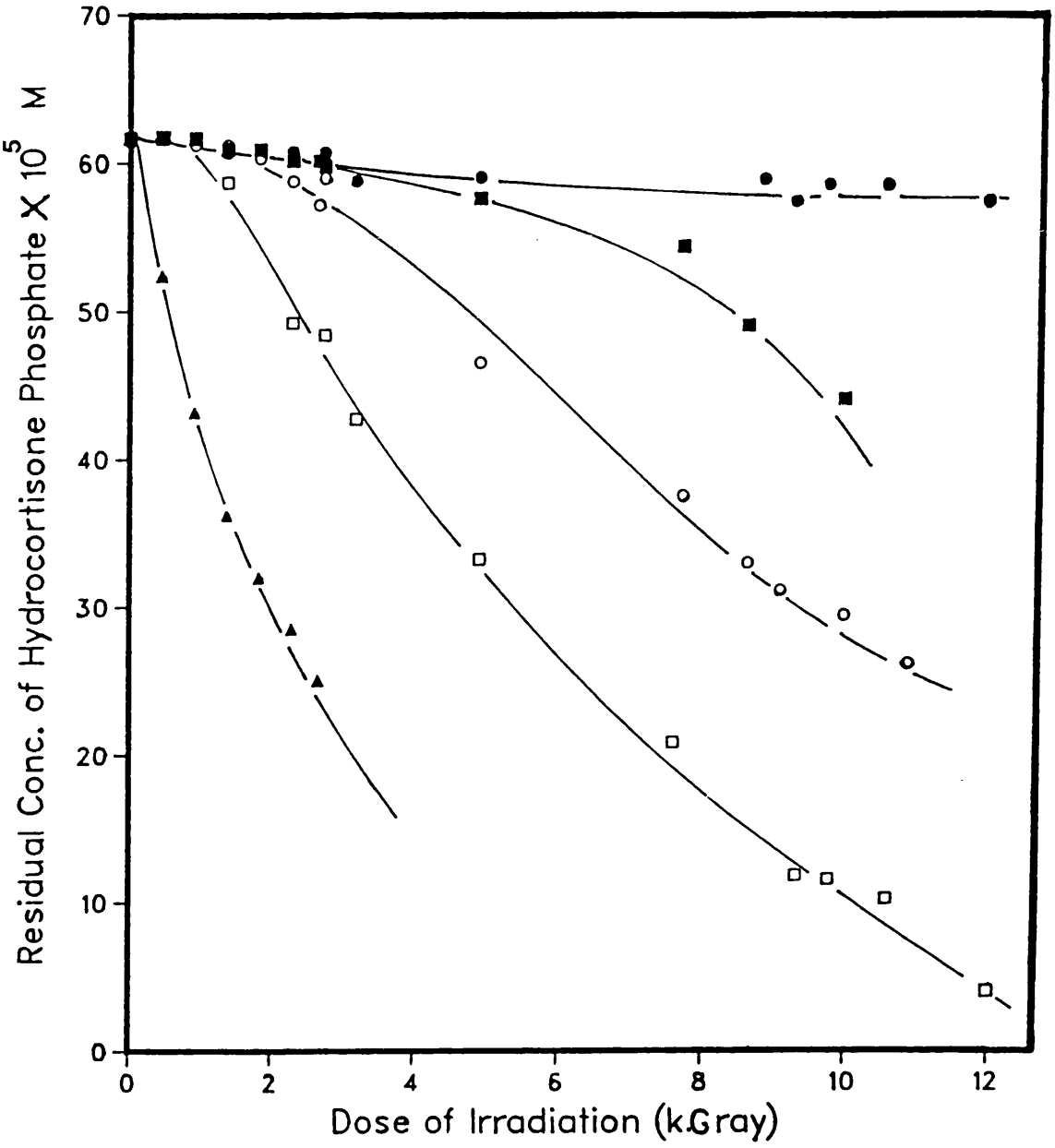


Fig. 3.4.7 The Effect of Methanol/2-Propanol Mixtures on the Sensitivity of Hydrocortisone Phosphate in Water to Ionising Radiation

▲ Water
□ 1% v/v Methanol + 1% v/v 2-propanol
○ 2% v/v Methanol + 2% v/v 2-propanol
■ 4% v/v Methanol + 4% v/v 2-propanol
● 7.5% v/v Methanol + 7.5% v/v 2-propanol

Table 3.4.2 THE EFFECT OF METHANOL AND 2-PROPANOL CONTENT ON THE SENSITIVITY OF HYDROCORTISONE PHOSPHATE IN WATER TO IONISING RADIATION

PERCENTAGE OF METHANOL OR 2-PROPANOL (v/v)	INITIAL REACTION RATE x 10^5 mol l^{-1} K.Gy $^{-1}$	
	METHANOL	2-PROPANOL
0	-19.48	-19.48
2	- 8.79	- 3.10
15	- 1.00	- 0.546

Table 3.4.3 THE EFFECT OF MIXTURES OF METHANOL AND 2-PROPANOL ON THE SENSITIVITY OF HYDROCORTISONE PHOSPHATE IN WATER TO IONISING RADIATION

PERCENTAGE OF METHANOL + 2-PROPANOL (v/v)	INITIAL REACTION RATE x 10^5 mol l^{-1} K.Gy $^{-1}$
0	-19.48
1 + 1	- 5.70
2 + 2	- 2.95
4 + 4	- 0.858
7.5 + 7.5	- 0.331

3.4.2 The Effect of Oxygen on Sensitivity of Hydrocortisone, Hydrocortisone Acetate in Propylene Glycol and Hydrocortisone Phosphate in Water to Ionising Radiation

It has been reported that one of the suggested mechanisms of protection in radiolysis of organic systems is the "quenching effect"[74]. A quencher, such as oxygen, may promote distribution of the initially localised energy among vibrational-rotational degrees of freedom of neighbouring solvent molecules which consequently results in a molecular decomposition or rearrangement process[75]. This effect was also reported by Hayon[35] who concluded that oxygen was able to quench the excited water molecules and convert them to solvated electrons and hydroxyl radicals:

$$O_2 + H_2O^* \longrightarrow \bar{e}_{aq.} + \dot{O}H$$

At the same time, oxygen may act as a scavenger for the solvated electron giving superoxide anion $\bar{O}_2$ [27].

Method: 6.896×10^{-4}M; 6.18×10^{-4}M of hydrocortisone, hydrocortisone acetate solutions in propylene glycol and 6.166×10^{-4}M hydrocortisone phosphate solution in water were separately prepared. Each solution was divided into three portions:

I - The first portion was unbubbled.

II - The second portion was bubbled with oxygen for half an hour.

III - The third portion was bubbled with helium or nitrogen for half an hour.

2 ml samples of each solution were irradiated in small vessels with different doses of radiation and analysis according to the respective standard assay procedure for the residual corticosteroid concentration by reference to the unirradiated solutions. The residual corticosteroid concentrations are plotted against dose of radiation in figs. 3.4.8, 3.4.9 and 3.4.10.

It is evident from figs. 3.4.8 and 3.4.9 and table 3.4.4 that the removal of oxygen from the propylene glycol solutions by bubbling either helium or nitrogen results in an increase in the degradation of hydrocortisone and hydrocortisone acetate by radiation, whereas saturation of the solutions with oxygen results in greater stabilisation of the corticosteroid to radiation. This latter effect could be due to the removal of the organic radicals by oxygen through the formation of stable peroxide derivatives, thus protecting the corticosteroids from attack by these radicals.

In the case of aqueous solution of hydrocortisone phosphate, it is clear from table 3.4.5 and fig. 3.4.10 that oxygen has no effect on the sensitivity of the corticosteroid to radiation.

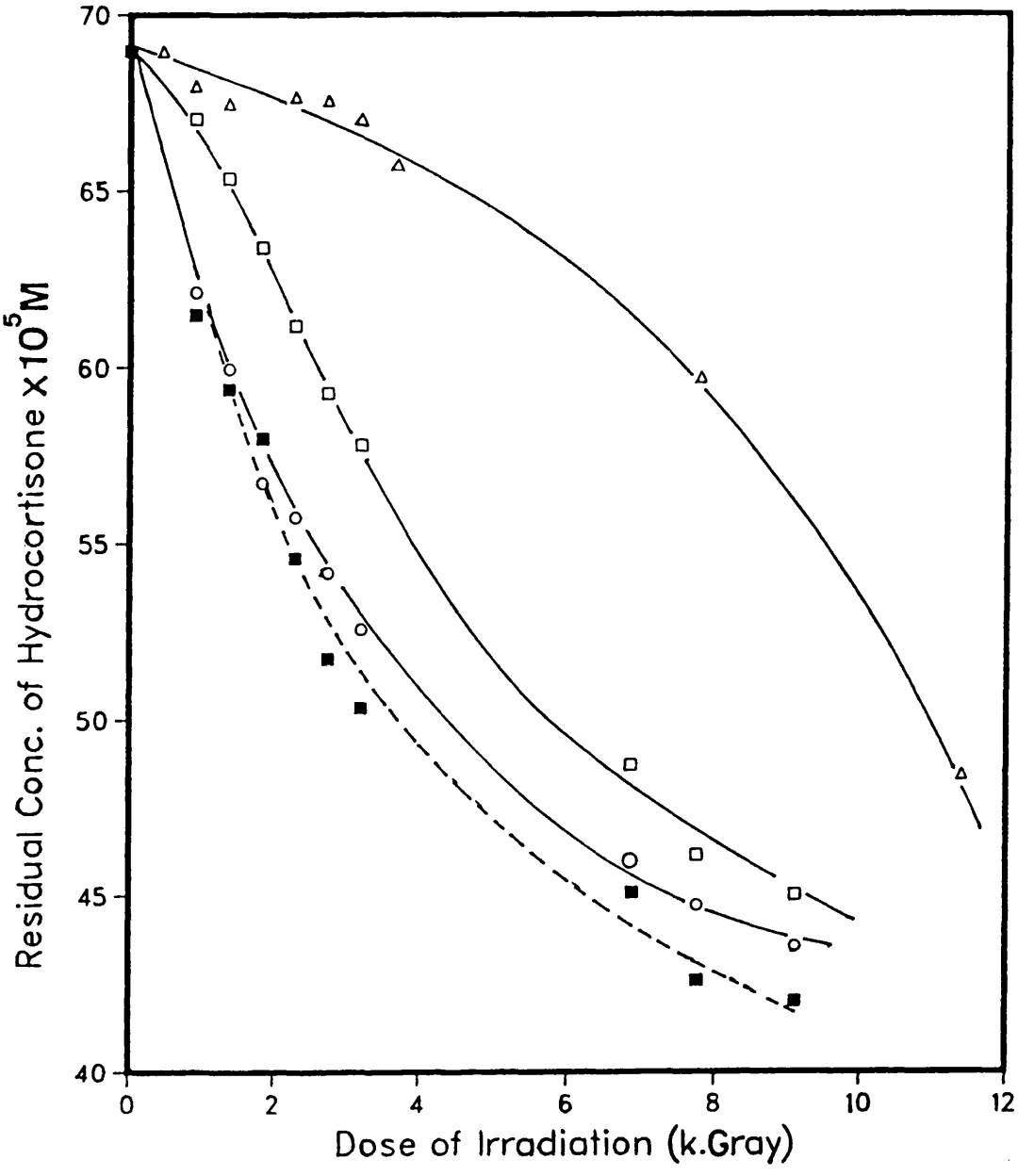


Fig. 3.4.8 The Effect of Oxygen on the Sensitivity of Hydrocortisone in Propylene Glycol to Ionising Radiation

□ Unbubbled

■ Helium

○ Nitrogen

Δ Oxygen

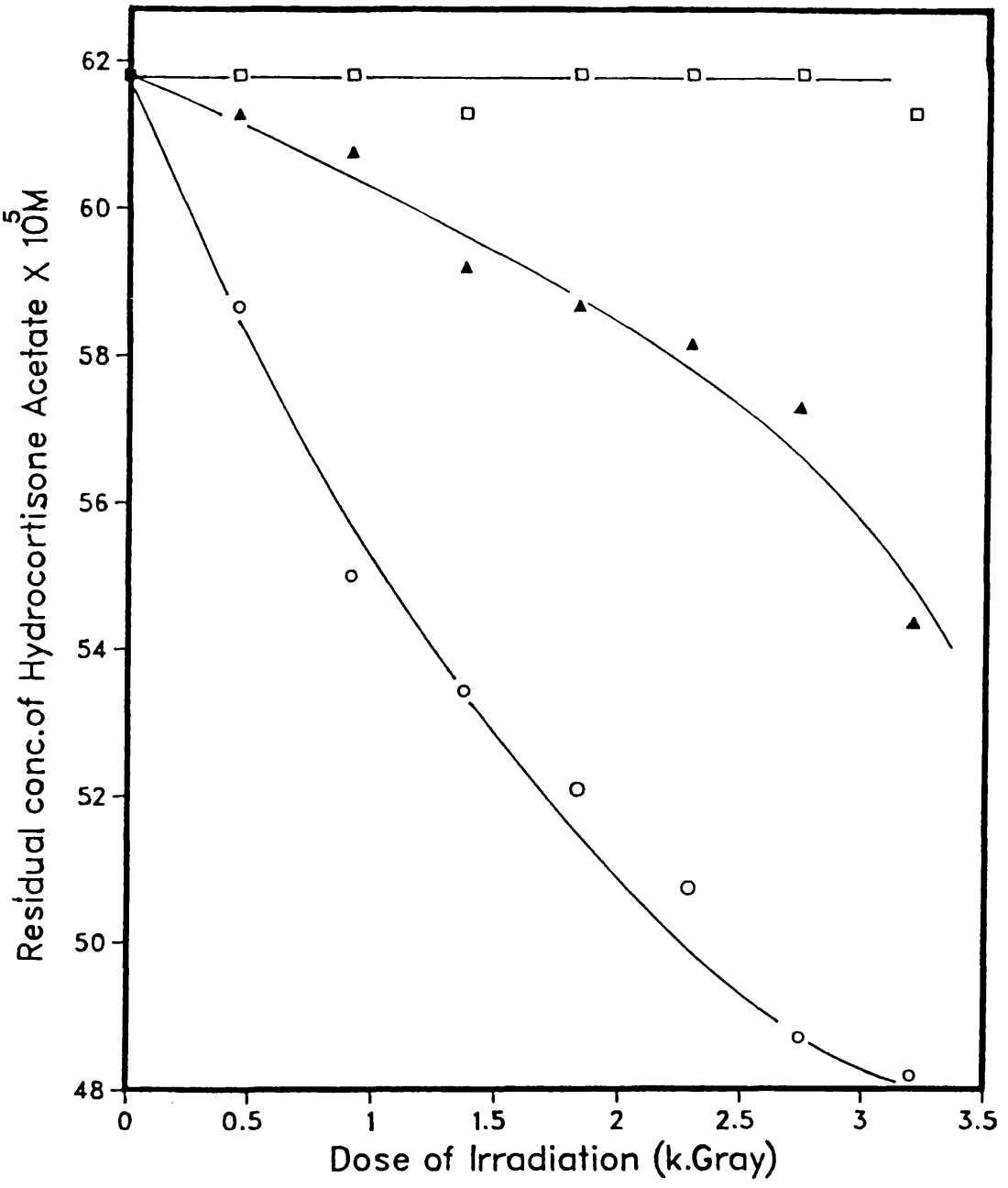


Fig. 3.4.9 The Effect of Oxygen on the Sensitivity of Hydrocortisone Acetate in Propylene Glycol to Ionising Radiation

▲ Unbubbled

O Helium

□ Oxygen

Table 3.4.4 THE EFFECT OF OXYGEN ON THE SENSITIVITY OF HYDROCORTISONE AND HYDROCORTISONE ACETATE IN PROPYLENE GLYCOL TO IONISING RADIATION

THE IRRADIATED SOLVENT	INITIAL REACTION RATE x 10^5 mol l^{-1} $K.Gy^{-1}$	
	HYDROCORTISONE	HYDROCORTISONE ACETATE
Unbubbled Propylene Glycol	-2.79	-1.98
Propylene Glycol bubbled with O_2	-0.713	-0.136
bubbled with helium	-5.73	-4.75
bubbled with N_2	-5.00	-

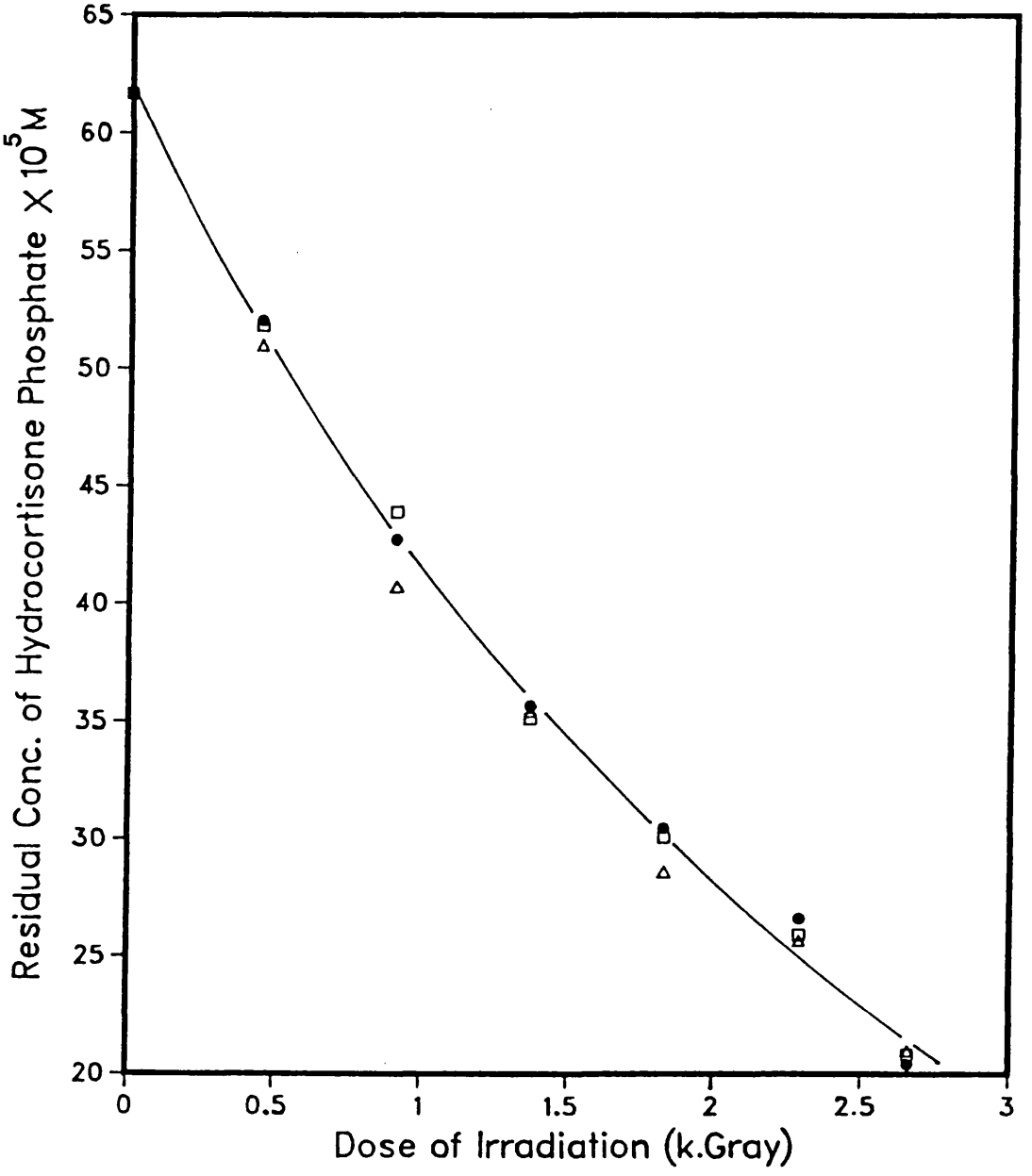


Fig. 3.4.10 The Effect of Oxygen on the Sensitivity of Hydrocortisone Phosphate in Water to Ionising Radiation

△ Unbubbled

● Helium

□ Oxygen

Table 3.4.5 THE EFFECT OF OXYGEN ON THE SENSITIVITY OF HYDROCORTISONE PHOSPHATE IN WATER TO IONISING RADIATION

THE IRRADIATED SOLVENT	INITIAL REACTION RATE x 10^5 mol l^{-1} $K.Gy^{-1}$	G^- VALUE
Unbubbled water	-19.13	1.85
Water bubbled with O_2	-19.43	1.87
Water bubbled with helium	-19.19	1.85

3.4.3 The Effect of Iodine on Sensitivity of Hydrocortisone and Hydrocortisone Acetate in Organic Solvents to Ionising Radiation

From studying the effect of methanol on sensitivity of hydrocortisone in organic solvents to radiation, it is evident that the organic radicals liberated from the solvents play a main role in attacking the corticosteroid. Therefore, it was decided to investigate the effect of iodine as an organic radical scavenger on the system.

Iodine can be dissolved in propylene glycol and glycerol either directly or by using methanol as a cosolvent. The concentration of iodine giving the maximum stability to hydrocortisone in propylene glycol to radiation was initially determined, then a comparison between the effect of iodine in the absence and presence of small amounts of methanol as a cosolvent was investigated in propylene glycol and glycerol.

Method: 6.896×10^{-4}M solutions of hydrocortisone were prepared using 10^{-4}M; 2×10^{-4}M and 10^{-3}M iodine in propylene glycol as solvents. 2 ml samples of each solution were irradiated in small vessels with different doses of radiation and analysed for the residual concentrations of hydrocortisone by reference to the unirradiated solutions. From the reaction rate of hydrocortisone presented in table 3.4.6 and plotted in fig. 3.4.11, it is noted that 10^{-3}M iodine gives the maximum stability of the corticosteroid in propylene glycol against radiation.

Using this concentration of iodine in propylene glycol, the same experiment was repeated to investigate the

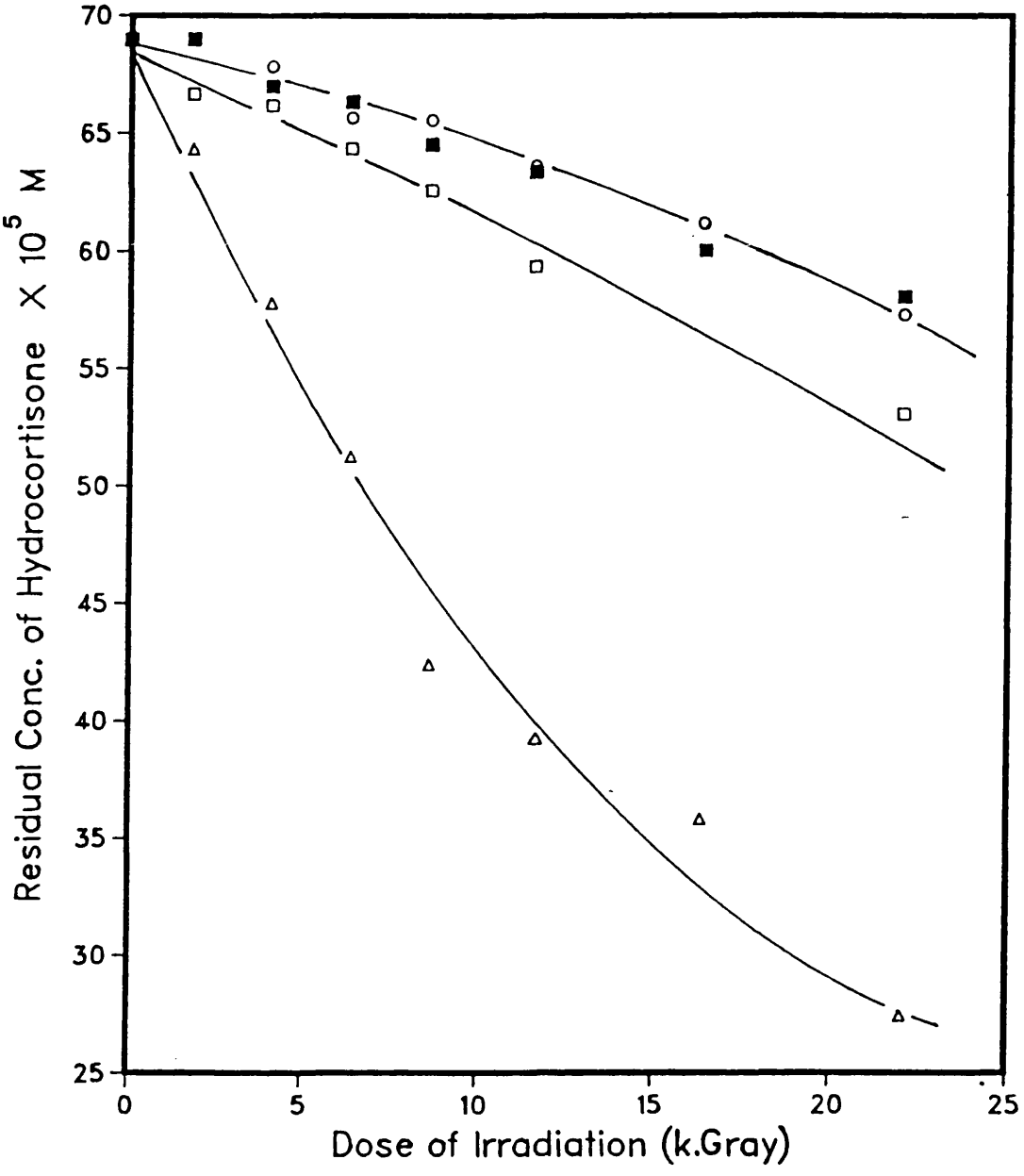


Fig. 3.4.11 The Effect of Iodine Concentration in Propylene Glycol on the Sensitivity of Hydrocortisone to Ionising Radiation

△ Propylene Glycol

□ 1×10^{-4}M I_2

■ 2×10^{-4}M I_2

○ 1×10^{-3}M I_2

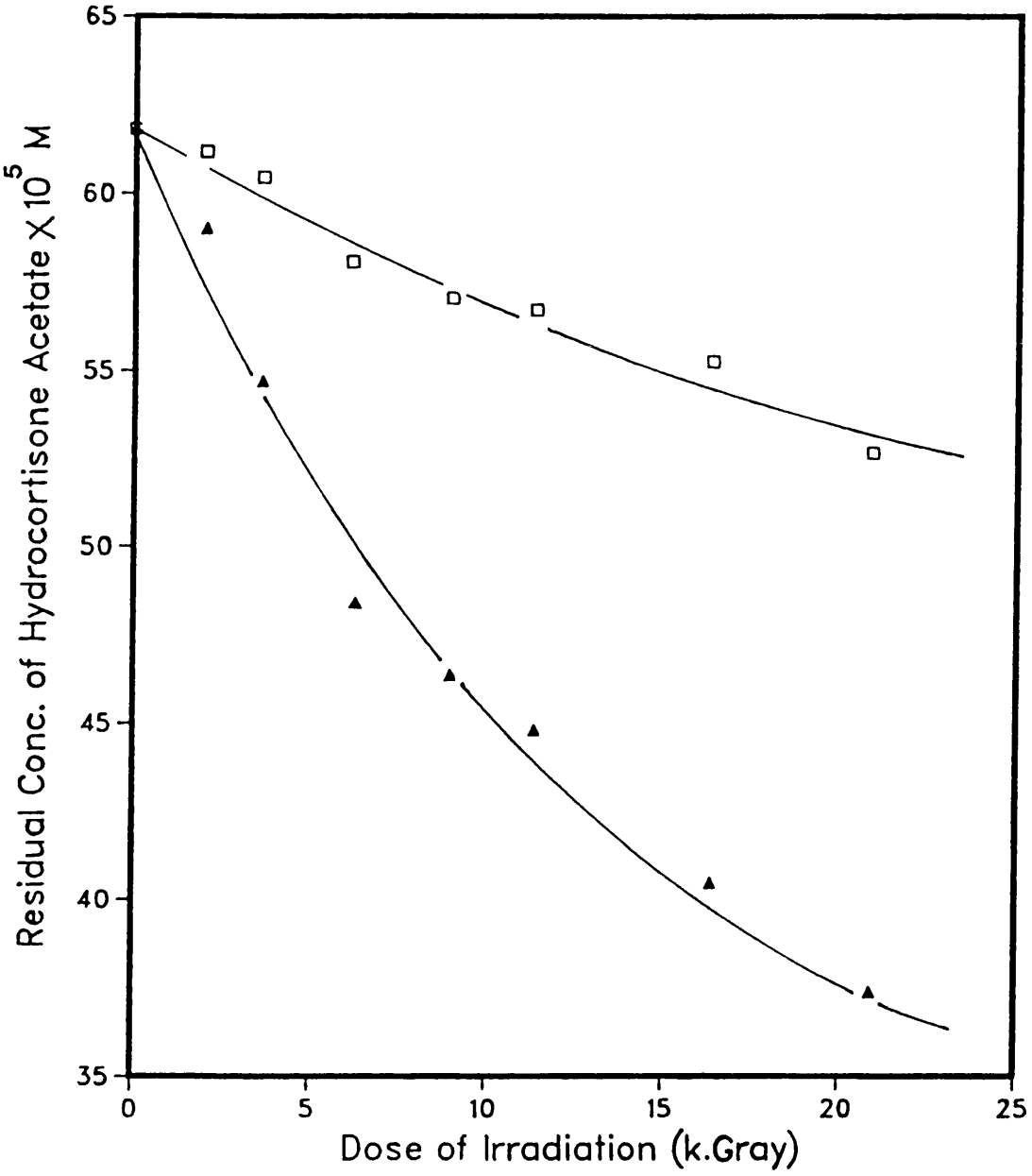


Fig. 3.4.12 The Effect of Iodine on the Sensitivity of Hydrocortisone Acetate in Propylene Glycol to Ionising Radiation

▲ Propylene Glycol

□ 1 x 10^{-3}M I_2

Table 3.4.6 THE EFFECT OF IODINE ON THE SENSITIVITY OF HYDROCORTISONE AND HYDROCORTISONE ACETATE IN PROPYLENE GLYCOL TO IONISING RADIATION

SOLVENT	INITIAL REACTION RATE x 10^5 mol l^{-1} $K.Gy^{-1}$	
	HYDROCORTISONE	HYDROCORTISONE ACETATE
Propylene Glycol	-2.79	-1.99
1 x 10^{-4}M Iodine in Propylene Glycol	-0.681	-
2 x 10^{-4}M Iodine in Propylene Glycol	-0.538	-
1 x 10^{-3}M Iodine in Propylene Glycol	-0.531	-0.433

sensitivity of hydrocortisone acetate in that system to ionising radiation and shown in fig.3.4.12, from which it is evident that iodine has the same stabilising effect on hydrocortisone acetate as hydrocortisone.

To investigate the effect of methanol as a co-solvent for iodine, 6.896×10^{-4}M solutions of hydrocortisone in propylene glycol and glycerol containing the following additives were prepared:

I - 10% v/v methanol.

II - 1×10^{-3}M iodine.

III - 1×10^{-3}M iodine dissolved in 10% v/v methanol.

2 ml samples of each solution were irradiated and analysed according to the standard assay procedure for the residual concentrations of hydrocortisone which are plotted against the dose of radiation in fig. 3.4.13 and 3.4.14.

From the calculated reaction rate presented in table 3.4.7 it is obvious that iodine has a significant stabilising effect on hydrocortisone to radiation in both propylene glycol and glycerol. However, the use of methanol as a cosolvent for the iodine results in a reduction in the apparent efficiency of iodine as a scavenger for the organic radicals.

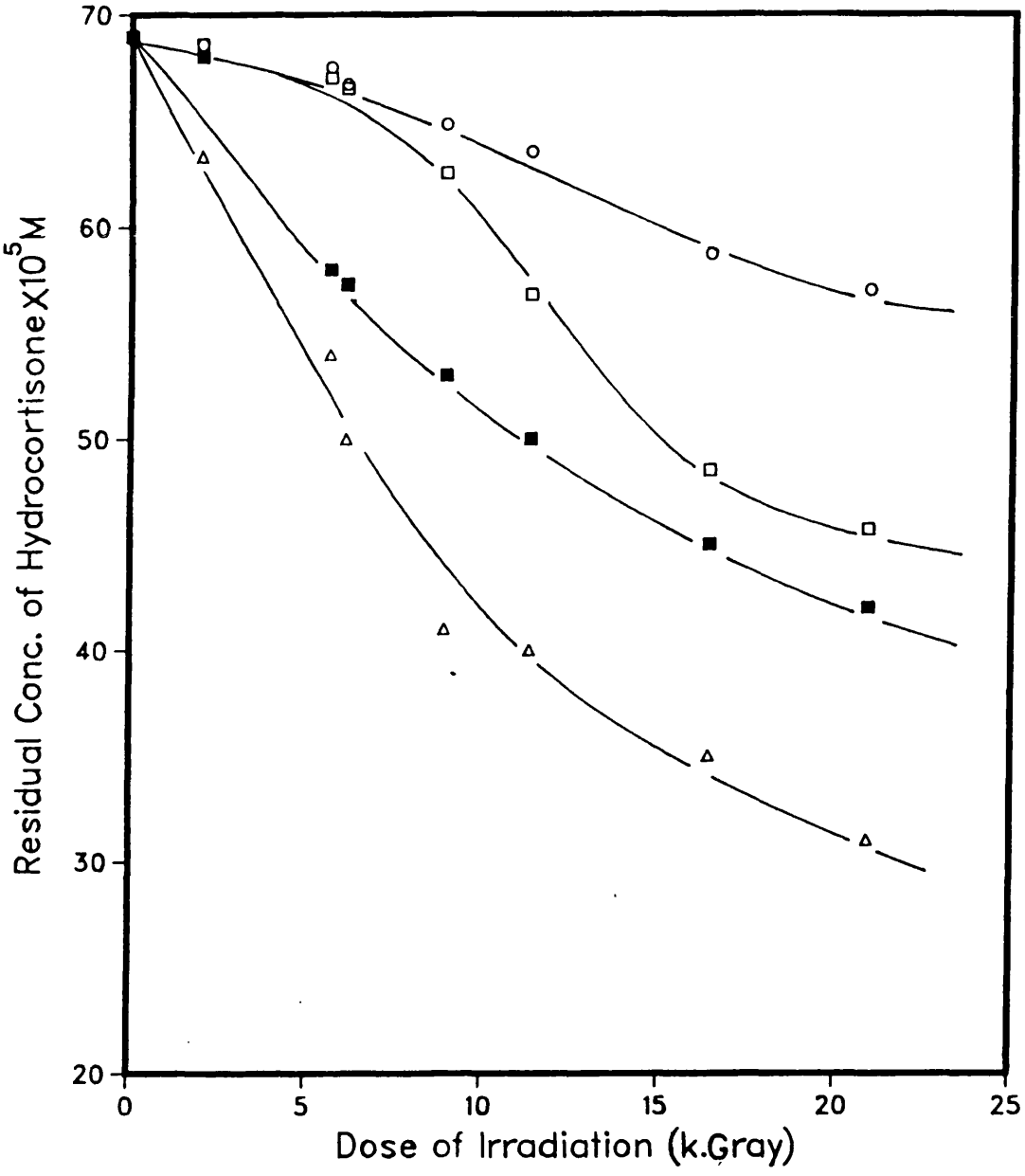


Fig. 3.4.13 The Effect of Methanol as a Cosolvent for Iodine on the Sensitivity of Hydrocortisone in Propylene Glycol to Ionising Radiation

- ○ 1 x 10^{-3}M I_2
- □ 10% v/v Methanol + 1 x 10^{-3}M I_2
- ■ 10% v/v Methanol
- △ Propylene Glycol

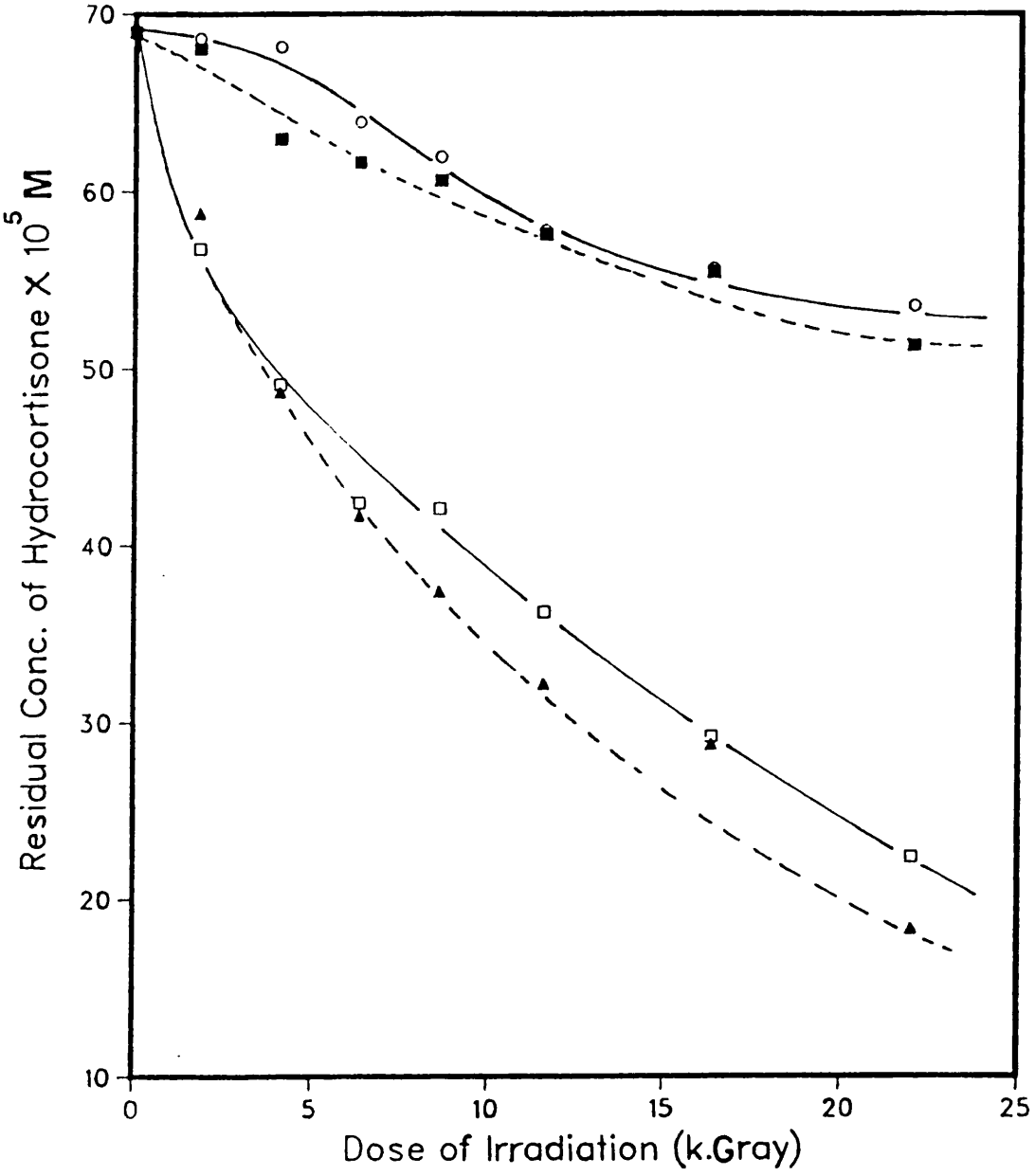


Fig. 3.4.14 The Effect of Iodine on the Sensitivity of Hydrocortisone in Glycerol to Ionising Radiation

▲ In Glycerol

□ 10% v/v Methanol

■ 10% v/v Methanol and 1 x 10^{-3}M I_2

○ 1 x 10^{-3}M I_2

Table 3.4.7 THE EFFECT OF 10^{-3}M IODINE ON THE SENSITIVITY OF HYDROCORTISONE IN PROPYLENE GLYCOL AND GLYCEROL IN PRESENCE AND ABSENCE OF 10% V/V METHANOL AS A COSOLVENT

ADDITIVES TO THE SOLVENT	INITIAL REACTION RATE x 10^5 mol.l^{-1} K.Gy^{-1}	
	PROPYLENE GLYCOL	GLYCEROL
0	-2.79	-3.17
10% v/v Methanol	-1.85	-2.69
10^{-3}M Iodine	-0.531	-0.920
10% v/v Methanol + 10^{-3}M Iodine	-1.25	-0.991

3.4.4 The Effect of Iodine on Sensitivity of Hydrocortisone Phosphate in Water to Ionising Radiation

After studying the effect of iodine in organic solvents it was decided to extend the investigation to the aqueous solution of the corticosteroid where the reactive radiolytic products of water are mainly hydrogen atoms and hydroxyl radicals. To dissolve iodine in water, it is necessary to use potassium iodide, to aid dissolution, or methanol as a cosolvent. Initially it was necessary to investigate the effect of KI alone and the concentration giving the maximum effect on sensitivity of hydrocortisone phosphate in water to ionising radiation. Then a comparison between the effect of iodine in the presence of KI and methanol was carried out.

Method: 6.166×10^{-4}M solutions of hydrocortisone phosphate in water containing 10^{-4}M; 10^{-3}M; 1.92×10^{-3}M and 3.01×10^{-3}M potassium iodide were prepared. 2 ml samples of each solution were irradiated with different doses of radiation and analysed for the residual concentrations of hydrocortisone phosphate. The reaction rate of hydrocortisone phosphate in each solution was calculated, presented in table 3.4.8 and plotted against the concentration of KI in fig. 3.4.15, from which it can be noted that 3.01×10^{-3}M KI gives the maximum stabilisation of hydrocortisone phosphate to radiation. Therefore, it was decided to use 3.01×10^{-3}M aqueous solution of KI as a solvent for iodine to study its effect on the corticosteroid sensitivity to radiation.

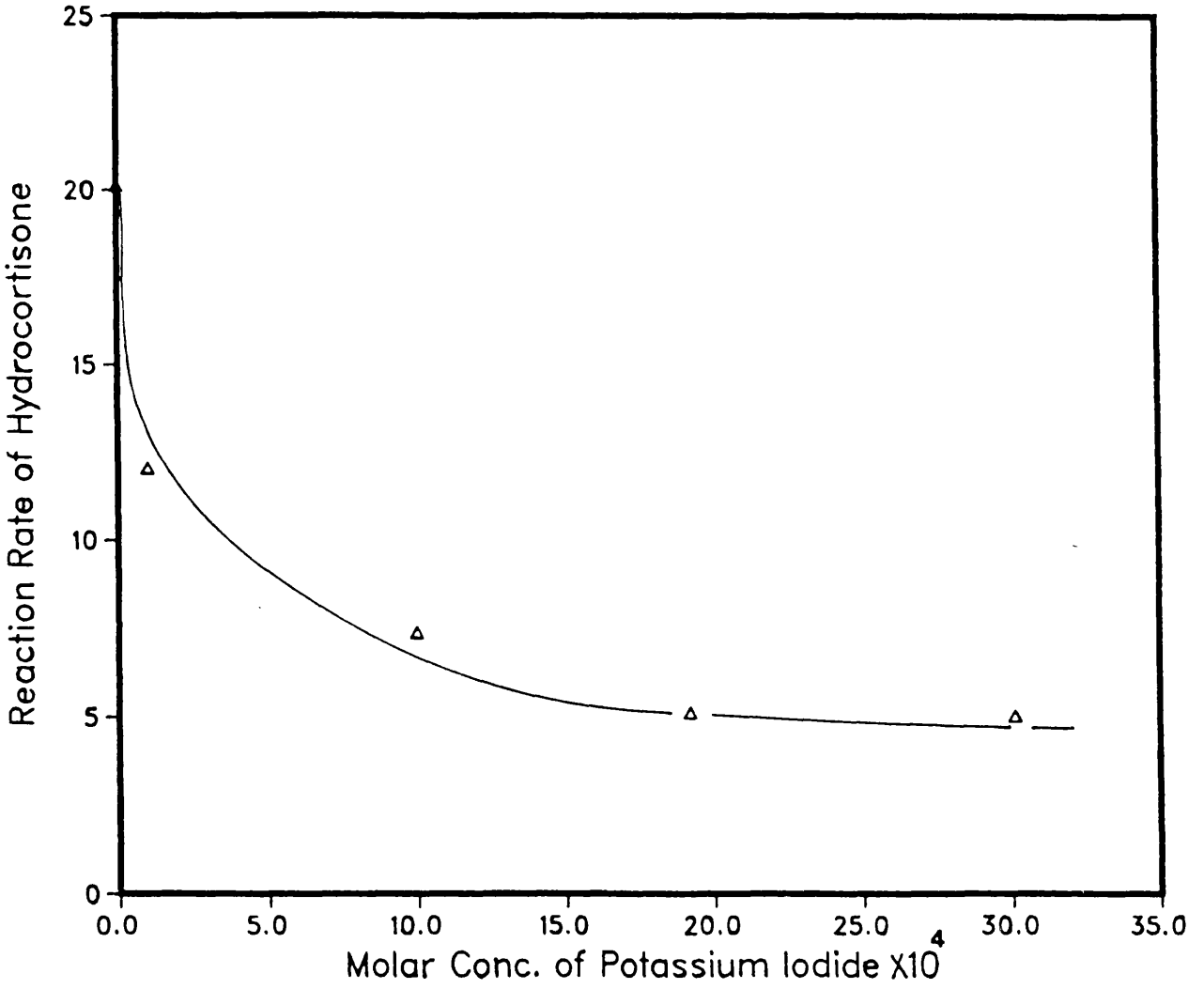


Fig. 3.4.15 The Effect of Different Concentrations of Potassium Iodide on the Reaction Rate of Hydrocortisone Phosphate in Water by Ionising Radiation

To compare the effect of iodine in the presence of KI and methanol, 6.166×10^{-4}M solutions of hydrocortisone phosphate in water containing the following additives were prepared:

I- 6×10^{-4}M I_2 and 3.01×10^{-3}M KI.

II- 3.01×10^{-3}M KI.

III- 2% v/v Methanol.

IV- 6×10^{-4}M I_2 and 2% v/v methanol.

2 ml samples of each solution were irradiated with different doses of radiation, and analysed as usual for the residual concentrations of hydrocortisone phosphate which are plotted against dose of radiation in fig. 3.4.16. Calculating the reaction rate of hydrocortisone phosphate in each solution as shown in table 3.4.9, it is evident that iodine is more effective as a stabiliser for the corticosteroid to radiation in KI than in methanol. The suggested role of iodine in stabilising hydrocortisone phosphate to radiation in aqueous solution could be due to the capture of solvated electron from the solution and the consequent formation of iodide ion I^- which in turn reacts with $\dot{O}H$ radical as follows[157]:

$$I_2 + \bar{e} \longrightarrow I_2^-$$

$$I^- + \dot{O}H \longrightarrow \bar{O}H + I^\cdot$$

$$2I^\cdot \longrightarrow I_2$$

To test this hypothesis, combinations of KI and iodine of different proportions were prepared and their effect compared to that of equivalent concentrations of KI alone.

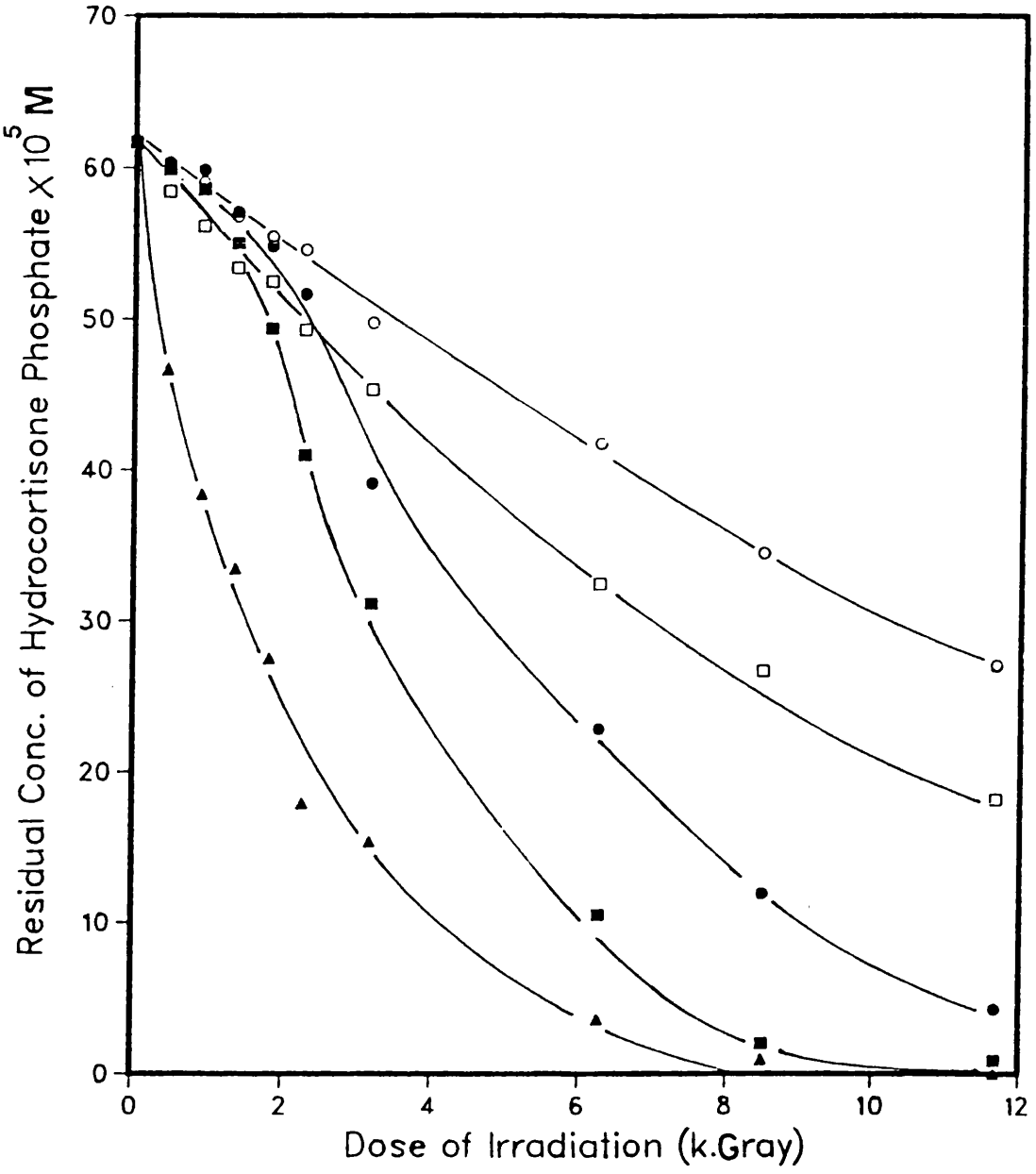


Fig. 3.4.16 The Effect of Iodine on the Sensitivity of Hydrocortisone Phosphate in Water to Ionising Radiation using Methanol and KI to Dissolve Iodine

▲ Water

■ 2% V/V Methanol

□ 3.01 x 10^{-3}M KI

● 2% V/V Methanol + 6 x 10^{-4}M I_2

O 3.01 x 10^{-3}M KI + 6 x 10^{-4}M I_2

Table 3.4.8 THE EFFECT OF CONCENTRATION OF POTASSIUM IODIDE ON SENSITIVITY OF HYDROCORTISONE PHOSPHATE IN WATER TO IONISING RADIATION

MOLAR CONCENTRATION OF KI	INITIAL REACTION RATE x 10^5 mol l^{-1} $K.Gy^{-1}$	G^- VALUE
0	-20.12	1.94
1×10^{-4}	-12.07	1.16
1×10^{-3}	- 7.43	0.717
1.92×10^{-3}	- 5.13	0.495
3.01×10^{-3}	- 5.04	0.486

Table 3.4.9 THE EFFECT OF IODINE ON SENSITIVITY OF HYDROCORTISONE PHOSPHATE IN WATER TO IONISING RADIATION USING 2% V/V METHANOL AND 3.01×10^{-3}M KI TO AID DISSOLUTION OF IODINE

ADDITIVES TO THE SOLVENT	INITIAL REACTION RATE x 10^5 mol l^{-1} $K.Gy^{-1}$	G^- VALUE
0	-20.12	1.94
3.01×10^{-3}M KI	- 5.04	0.486
6×10^{-4}M I_2 + 3.01×10^{-3}MKI	- 3.66	0.353
2% V/V Methanol	- 8.79	0.848
6×10^{-4}M I_2+ 2%v/V Methanol	- 6.29	0.607

Method: Two groups of 6.166 x 10^{-4}M aqueous solutions of hydrocortisone phosphate were prepared. The first group consisted of three solutions containing three different concentrations of KI, 10^{-4}M; 10^{-3}M and 1.92 x 10^{-3}M. The second group consisted of three solutions containing three different combinations of I_2 and KI which are supposed to produce total concentrations of KI after radiation equivalent to those in the first group, these combinations were:

5 x 10^{-5}M KI + 2.38 x 10^{-5}M I_2,

4.9 x 10^{-4}M KI + 2.38 x 10^{4}M I_2, and

10^{-3}M KI + 4.76 x 10^{-4}M I_2.

2 ml samples of each of these solutions were irradiated with different doses of radiation and analysed as usual for the residual concentrations of hydrocortisone phosphate. Comparing the reaction rate of hydrocortisone phosphate in presence of KI alone and its mixture with iodine, in table 3.4.10, it is evident that both systems had nearly the same reaction rates. This would support the hypothesis that iodine initially captures the solvated electron, and the produced iodide ion I^- acts as a scavenger for the hydroxyl radical.

Table 3.4.10 THE EFFECT OF DIFFERENT COMBINATIONS OF POTASSIUM IODIDE AND IODINE ON THE SENSITIVITY OF HYDROCORTISONE PHOSPHATE IN WATER TO IONISING RADIATION

ADDITIVES TO THE SOLVENT	INITIAL REACTION RATE $\times 10^5$ mol l^{-1} $K.Gy^{-1}$	G^- VALUE
1×10^{-4}M KI	-12.07	1.164
5×10^{-5}M KI + 2.38×10^{-5}M I_2	-12.41	1.197
1×10^{-3}M KI	- 7.43	0.717
5×10^{-4}M KI + 2.38×10^{-4}M I_2	- 7.59	0.732
1.92×10^{-3}M KI	- 5.13	0.495
9.5×10^{-4}M KI+4.76×10^{-4}M I_2	- 5.60	0.540

3.5 Effect of Surfactants on the Sensitivity of Corticosteroids in Aqueous Solutions to Ionising Radiation

In pharmaceutical formulations the influence of a surfactant on the stability of the product is generally secondary to its main purpose as a solubilising and dispersing agent, but surfactants may be used to stabilise labile pharmaceuticals. For example, the transfer of a reactive solute into a micelle results in a considerable change in the environment of the solute molecules, from an aqueous to a relatively non-polar medium, depending on the depth of location in the micelle. The micellar environment is sufficiently different from the simple aqueous environment that reaction rates may sometimes be dramatically changed[158]. However, in some micelles the surface characteristics will be such that there is a concentration of species, responsible for degrading a drug, which would therefore result in a more rapid breakdown of the solute molecules than in simple aqueous solution[158].

The objective in this section of the investigation was to explore the influence of different types of surfactants on the sensitivity of hydrocortisone and hydrocortisone phosphate in aqueous solution to γ-radiation. The cationic, anionic, non-ionic surfactants chosen for this purpose were cetrimide (CTAB), sodium lauryl sulphate (NaLS) and cetomacrogol 1000 respectively.

Analysis of Corticosteroids in Presence of Surfactants

A standard assay procedure was determined for hydrocortisone and hydrocortisone phosphate using a column of 15cm x0.46cm packed with spherisorb ODS of 5 microns particle size as the stationary phase. Preliminary experiments using this column indicated that the best separation for hydrocortisone from its internal standard hydrocortisone phosphate was achieved by a mobile phase consisting of methanol : 0.09M KH_2 PO_4 (55 : 45). For simplicity, it was decided to use hydrocortisone as an internal standard for hydrocortisone phosphate using the same mobile phase. The flow rate used for both these separations was 1 ml/minute and the sample detection was carried out as usual by u.v. absorption at 248 nm.

Using these preliminary findings, calibration curves for a range of concentrations of the two corticosteroids in the presence of different concentrations of the three surfactants, were carried out to assess the reproducibility of such an assay procedure.

3.5.1 Calibration Curves of Hydrocortisone and Hydrocortisone Phosphate in Presence of Surfactants

Aqueous solutions of 1 x 10^{-2}M; 4 x 10^{-2}M and 1 x 10^{-3}M of CTAB, NaLS and cetomacrogol 1000 respectively were prepared, and stored at 30°C in volumetric flasks which had been aged by storing the surfactant solutions for 24 hours, rinsed gently with distilled water and dried.

The following concentrations of hydrocortisone and hydrocortisone phosphate in aqueous solutions of 8 x 10^{-5}M; 1 x 10^{-4}M; 4 x 10^{-4}M; 1 x 10^{-3}M and 2 x 10^{-3}M of CTAB were prepared:

Hydrocortisone:

0.827 x 10^{-4}M; 1.034 x 10^{-4}M; 1.041 x 10^{-4}M; 1.655 x 10^{-4}M and 2.068 x 10^{-4}M.

Hydrocortisone Phosphate:

2.055 x 10^{-4}M; 2.877 x 10^{-4}M; 3.699 x 10^{-4}M; 4.521 x 10^{-4}M and 5.138 x 10^{-4}M

2.055 x 10^{-4}M and 2.068 x 10^{-4}M aqueous solutions of hydrocortisone phosphate and hydrocortisone were prepared to be used as internal standard for hydrocortisone and hydrocortisone phosphate respectively. 1 ml of each concentration of hydrocortisone or hydrocortisone phosphate was mixed with 1 ml of the respective internal standard in a 10 ml volumetric flask and diluted to 10 ml with water. 3 x 20 µl samples of each mixture were injected by means of a loop valve on to the HPLC column and the mean ratio of

peak height of the respective corticosteroid to its internal standard, measured at 248 nm., was calculated. The experiment was repeated and the mean results were submitted to a computerised least squares regression analysis giving the slopes, intercepts, standard deviations and correlation coefficients.

The experiment was repeated twice for each of the following concentrations of NaLS and cetomacrogol 1000:

NaLS:

1×10^{-5}M; 1×10^{-4}M; 1×10^{-3}M; 5×10^{-3}M and 4×10^{-2}M.

Cetomacrogol 1000;

8×10^{-6}M; 4×10^{-5}M; 8×10^{-5}M; 4×10^{-4}M and 8×10^{-4}M.

The obtained mean results are presented in tables 3.5.1 and 3.5.2 from which it is evident that the assay procedure for both corticosteroids is reproducible and the surfactants do not interfere with the assays reproducibility. It was decided therefore to use this assay procedure for the two corticosteroids in determining the effect of surfactants on the sensitivity of the drugs to γ-radiation below and above the surfactant concentration which normally produces micellisation.

Table 3.5.1 DATA FOR CALIBRATION CURVES FOR HPLC ASSAY OF HYDROCORTISONE IN AQUEOUS SOLUTIONS OF CTAB, NaLS AND CETOMACROGOL 1000

MOLAR CONCENTRATION OF SURFACTANT	SLOPE	STANDARD DEVIATION OF SLOPE	INTERCEPT	STANDARD DEVIATION OF INTERCEPT	t-RATIO ($\frac{\text{SLOPE}}{\text{S.D.}}$)	CORRELATION COEFFICIENT
0	2330.91	58.05	0.01797	0.08131	40.15	0.9998
CTAB						
8×10^{-5}	2380.60	61.51	-0.00673	0.02251	38.70	0.9989
1×10^{-4}	2424.09	96.92	-0.01693	0.03547	25.01	0.9985
4×10^{-4}	2396.90	117.5	-0.00552	0.04300	20.40	0.9959
1×10^{-3}	2314.44	78.03	0.01077	0.02855	29.66	0.9987
2×10^{-3}	2299.04	42.34	0.00406	0.01549	54.29	0.9998
NaLS						
1×10^{-5}	2333.47	81.64	0.00047	0.02987	28.58	0.9960
1×10^{-4}	2285.40	74.55	0.01637	0.05470	30.65	0.9987
1×10^{-3}	2251.02	16.49	0.01215	0.00603	136.52	1.00

continued...../

Table 3.5.1. (continued)

MOLAR CONCENTRATION OF SURFACTANT	SLOPE	STANDARD DEVIATION OF SLOPE	INTERCEPT	STANDARD DEVIATION OF INTERCEPT	t-RATIO ($\frac{\text{SLOPE}}{\text{S.D.}}$)	CORRELATION COEFFICIENT
5×10^{-3}	2260.08	25.16	0.01035	0.00920	89.83	1.00
4×10^{-2}	2280.01	31.38	0.00876	0.01148	72.66	0.9999
Cetomacrogol 1000						
8×10^{-6}	2295.20	109.00	0.00117	0.03988	21.05	0.9955
4×10^{-5}	2291.80	89.91	0.01347	0.05487	25.48	0.9987
8×10^{-5}	2259.17	23.95	0.01205	0.00876	94.32	1.00
4×10^{-4}	2221.10	54.69	0.02406	0.02001	40.61	0.9987
8×10^{-4}	2320.78	83.80	0.00347	0.03066	27.69	0.9960

Table 3.5.2 DATA FOR CALIBRATION CURVES FOR HPLC ASSAY OF HYDROCORTISONE PHOSPHATE IN AQUEOUS SOLUTIONS OF CTAB, NaLS AND CETOMACROGOL 1000

MOLAR CONCENTRATION OF SURFACTANT	SLOPE	STANDARD DEVIATION OF SLOPE	INTERCEPT	STANDARD DEVIATION OF INTERCEPT	t-RATIO ($\frac{\text{SLOPE}}{\text{S.D.}}$)	CORRELATION COEFFICIENT
0	2239.90	28.19	0.00197	0.01157	79.45	0.9999
CTAB						
8×10^{-5}	2261.60	36.92	0.00519	0.01510	61.25	0.9998
1×10^{-4}	2268.10	77.57	0.00498	0.03172	29.24	0.9987
4×10^{-4}	2208.07	45.91	0.01699	0.01878	48.10	0.9997
1×10^{-3}	2273.76	32.05	0.00389	0.01311	70.95	0.9999
2×10^{-3}	2315.13	61.88	-0.00761	0.02531	37.41	0.9988
NaLS						
1×10^{-5}	2237.28	43.57	0.01019	0.01782	51.35	0.9999
1×10^{-4}	2257.54	27.08	0.01569	0.1108	83.36	1.00
1×10^{-3}	2272.14	34.00	0.01009	0.01390	66.82	0.9998

Continued...../

Table 3.5.2 (continued)

MOLAR CONCENTRATION OF SURFACTANT	SLOPE	STANDARD DEVIATION OF SLOPE	INTERCEPT	STANDARD DEVIATION OF INTERCEPT	t-RATIO $(\frac{\text{SLOPE}}{\text{S.D.}})$	CORRELATION COEFFICIENT
5×10^{-3}	2247.81	35.43	0.01389	0.01449	63.44	0.9998
4×10^{-2}	2304.58	37.01	0.00169	0.01514	62.26	0.9999
Cetomacrogol 1000						
8×10^{-6}	2285.11	18.93	0.00889	0.007740	120.74	1.00
4×10^{-5}	2242.12	47.98	0.02759	0.01962	46.73	0.9999
8×10^{-5}	2328.92	58.43	-0.00871	0.02390	39.86	0.9988
4×10^{-4}	2322.41	13.20	0.00869	0.00539	175.93	1.00
8×10^{-4}	2316.74	9.91	-0.00160	0.00405	233.69	1.00

3.5.2 Effect of CTAB, NaLS and Cetomacrogol 1000 on the Sensitivity of Hydrocortisone and Hydrocortisone Phosphate in Aqueous Solutions to γ-Radiation

Using the stock solutions of surfactants, the following aqueous concentrations containing 5.517×10^{-4}M Hydrocortisone were prepared:

CTAB:

4×10^{-5}M; 8×10^{-5}M; 1×10^{-4}M; 3×10^{-4}M, 6×10^{-4}M; 9×10^{-4}M; 2×10^{-3}M; 4×10^{-3}M and 6×10^{-3}M.

NaLS:

4×10^{-5}M; 8×10^{-5}M; 4×10^{-4}M; 8×10^{-4}M; 2×10^{-3}M; 4×10^{-3}M; 8×10^{-3}M; 2×10^{-2}M and 4×10^{-2}M.

Cetomacrogol 1000:

2×10^{-6}M; 4×10^{-6}M; 8×10^{-6}M; 2×10^{-5}M; 4×10^{-5}M; 8×10^{-5}M; 2×10^{-4}M; 4×10^{-4}M and 8×10^{-4}M.

2 ml samples of each of these solutions were irradiated in small vessels with different doses of radiation, analysed according to the standard assay procedure for the residual concentrations of hydrocortisone and hydrocortisone phosphate and the reaction rate of the corticosteroid in each solution was calculated by a computerised least squares regression analysis. The experiment was repeated and the mean G^- values were then calculated, presented in tables 3.5.3, 3.5.4 and 3.5.5 and plotted against the molar concentration of the surfactant, as in fig. 3.5.1.

The same experiments were repeated using 5.549×10^{-4}M hydrocortisone phosphate instead of hydrocortisone, the

calculated reaction rates and the mean G^- values are presented in tables 3.5.3, 3.5.4 and 3.5.5 and plotted against the molar concentration of the surfactant as shown in fig. 3.5.2.

From plots shown in fig. 3.5.1 and 3.5.2 it is clear that all the three types of surfactants have a considerable stabilising effect on hydrocortisone and hydrocortisone phosphate against radiation. These stabilising effects are in the order NaLS > CTAB > Cetomacrogol 1000.

As the results, shown in fig. 3.5.1 and 3.5.2, represent the total effect of the radiolytic products of water ($\dot{H}$, $\dot{O}H$ and $\bar{e}$ aq.), it was decided therefore to investigate the effect of the individual radiolytic products of water on both corticosteroids in the presence of the three types of surfactants.

Table 3.5.3 REACTION RATES AND G^- VALUES OF HYDROCORTISONE AND HYDROCORTISONE PHOSPHATE DEGRADATION BY GAMMA-RADIATION IN AQUEOUS SOLUTIONS OF CTAB

MOLAR CONCENTRATION OF SURFACTANT	HYDROCORTISONE		HYDROCORTISONE PHOSPHATE	
	REACTION RATE	G^- VALUE	REACTION RATE	G^- VALUE
0	-17.77	1.715	-20.82	2.00
4×10^{-5}	-17.11	1.651	-18.15	1.75
8×10^{-5}	-16.79	1.620	-15.86	1.53
1×10^{-4}	-15.45	1.491	-15.37	1.48
3×10^{-4}	-11.51	1.111	-14.82	1.43
6×10^{-4}	- 9.86	0.951	-12.49	1.20
9×10^{-4}	-10.09	0.973	- 9.80	0.945
2×10^{-3}	-10.30	0.994	- 8.03	0.774
4×10^{-3}	-10.20	0.984	- 7.07	0.682
6×10^{-3}	- 9.54	0.920	- 5.71	0.551

Table 3.5.4 REACTION RATES AND G^- VALUES OF HYDROCORTISONE AND HYDROCORTISONE PHOSPHATE DEGRADATION BY GAMMA-RADIATION IN AQUEOUS SOLUTIONS OF NaLS

MOLAR CONCENTRATION OF SURFACTANT	HYDROCORTISONE		HYDROCORTISONE PHOSPHATE	
	REACTION RATE	G^- VALUE	REACTION RATE	G^- VALUE
0	-17.73	1.711	-20.79	2.00
4×10^{-5}	-14.73	1.421	-16.15	1.558
8×10^{-5}	-12.38	1.194	-15.02	1.449
4×10^{-4}	-10.67	1.029	-11.42	1.102
8×10^{-4}	- 9.22	0.889	-10.17	0.981
2×10^{-3}	- 6.49	0.626	- 6.14	0.592
4×10^{-3}	- 6.52	0.629	- 4.40	0.424
8×10^{-3}	- 5.59	0.539	- 4.46	0.430
2×10^{-2}	- 6.40	0.617	- 4.00	0.386
4×10^{-2}	- 4.67	0.450	- 2.93	0.282

Table 3.5.5 REACTION RATES AND G^- VALUES OF HYDROCORTISONE AND HYDROCORTISONE PHOSPHATE DEGRADATION BY GAMMA-RADIATION IN AQUEOUS SOLUTIONS OF CETOMACROGOL 1000

MOLAR CONCENTRATION OF SURFACTANT	HYDROCORTISONE		HYDROCORTISONE PHOSPHATE	
	REACTION RATE	G^- VALUE	REACTION RATE	G^- VALUE
0	-17.69	1.707	-20.17	1.946
2×10^{-6}	-16.90	1.630	-20.04	1.933
4×10^{-6}	-15.84	1.52	-19.12	1.845
8×10^{-6}	-13.91	1.342	-18.92	1.825
2×10^{-5}	-15.61	1.506	-19.64	1.895
4×10^{-5}	-15.84	1.528	-19.41	1.873
8×10^{-5}	-14.99	1.446	-18.44	1.779
2×10^{-4}	-13.93	1.344	-17.55	1.693
4×10^{-4}	-15.06	1.453	-15.92	1.536
8×10^{-4}	-13.45	1.298	-14.84	1.432

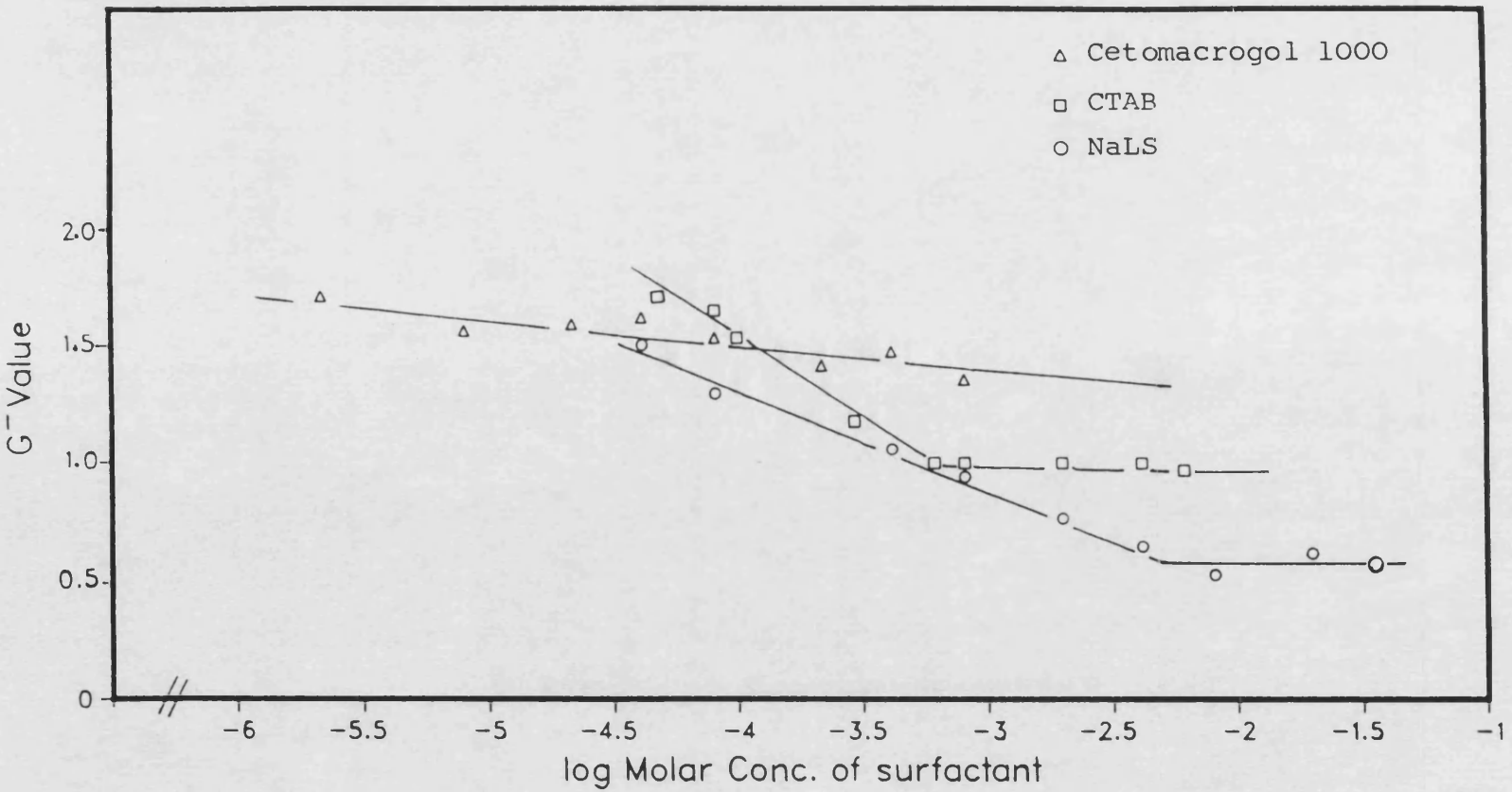


Fig. 3.5.1 Plots of G^- Value of Hydrocortisone Degradation in Aqueous Solutions by Ionising Radiation Against Log Molar Concentration of Surfactants

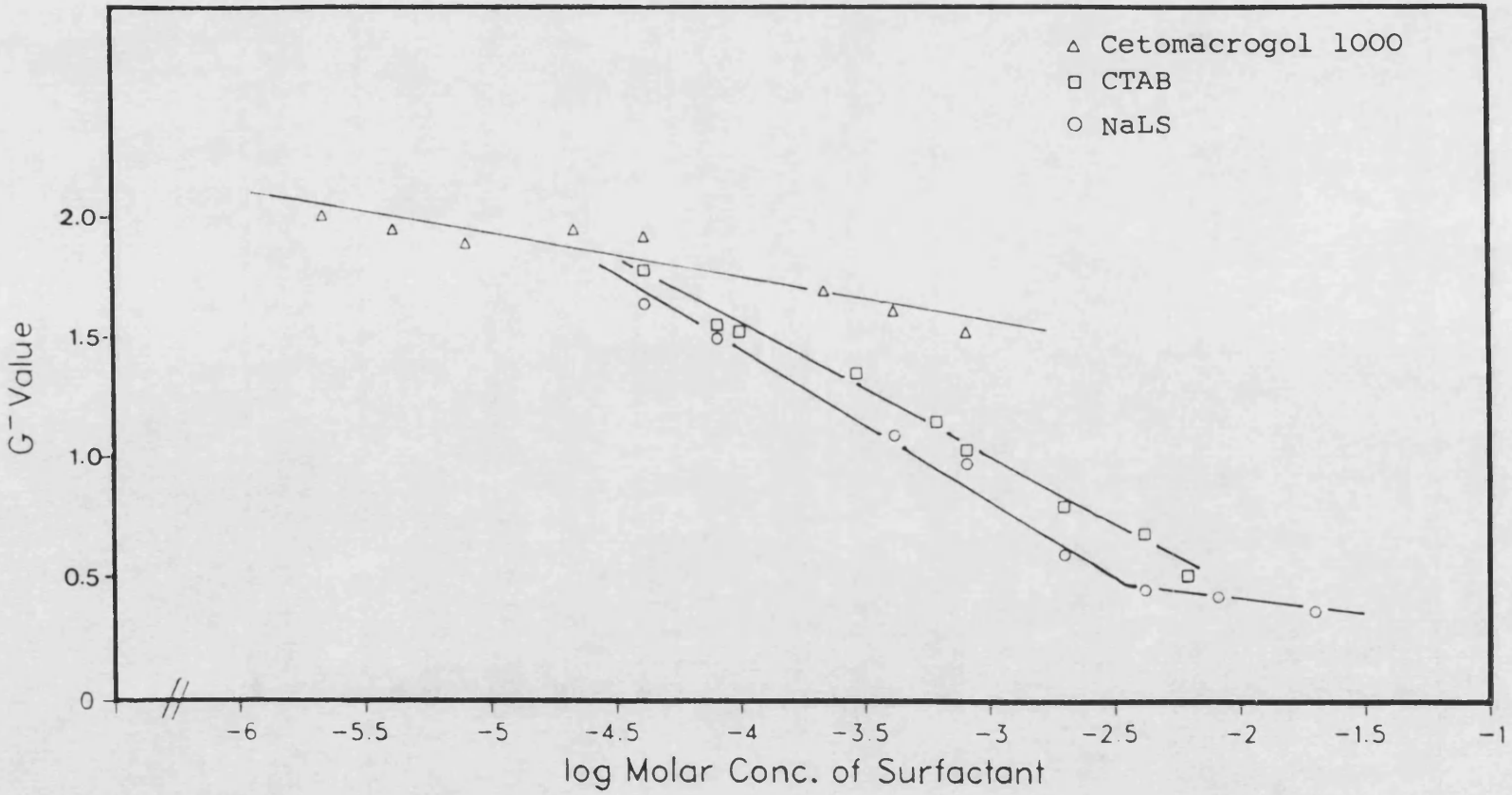


Fig. 3.5.2 Plots of G^- Value of Hydrocortisone Phosphate Degradation in Aqueous Solutions by Ionising Radiation Against Log Molar Concentration of Surfactants

3.5.3 Determination of Solubilisation Sites of Hydrocortisone in the Surfactants

Advantage has been taken of changes in the ultra-violet absorption maxima of several solubilisates as a function of solvent polarity in order to determine the mode of solubilisation and the position of the solubilisate in the micelle[159]. Resemblance of the absorption spectra of the solubilisate in the micellar phase to that in polar solvents is generally interpreted as implying a polar environment of the substrate in the micelle. Conversely, a similarity between the absorption spectrum in the micellar solution and that in a non-polar solvents is said to indicate that the substrate is solubilised in a hydrocarbon-like environment. The simplicity of absorption spectrophotometry has rendered this technique popular. Using this technique it was decided to investigate the site of solubilisation of hydrocortisone in the three surfactants.

Method:

5.517×10^{-4}M hydrocortisone solutions in 1×10^{-3}M, 1×10^{-2}M and 4×10^{-2}M of cetomacrogol 1000, CTAB and NaLS respectively were prepared. For the purpose of comparison with polar and non-polar solvents 5.517×10^{-4}M hydrocortisone solutions in water and in octanol were prepared. Using 1 cm, quartz matched cuvettes and Perkin Elmer-Lambda 3 uv/vis. spectrophotometer, the wavelength of maximum absorption of hydrocortisone in the surfactant solutions were determined three times using the

respective surfactant solutions without hydrocortisone as controls. Using the same cuvettes, the wavelength of maximum absorptions of hydrocortisone in water and octanol were determined. The obtained data are presented in table 3.5.6 and the ultraviolet spectra of all solutions are shown in fig. 3.5.3, from which, it can be noted that there is a shift in the λ_{max} of hydrocortisone in surfactant solutions from aqueous solution and this shift is the highest in the case of CTAB solution. Comparing the λ_{max} of hydrocortisone in surfactant solutions to that in aqueous and octanol solution it is clear that the λ_{max} of the corticosteroid in surfactant solutions shifts to a value near to that of octanol, the less polar solvent, which would suggest that the corticosteroid is surrounded by a more non-polar environment than water, but not to the extent of non-polarity of octanol. So it can be predicted that hydrocortisone is located in the polyoxyethylene shell of the cetomacrogol 1000 micelle, while possibly in the outer layer of the core in the case of NaLS and probably slightly deeper in the case of CTAB.

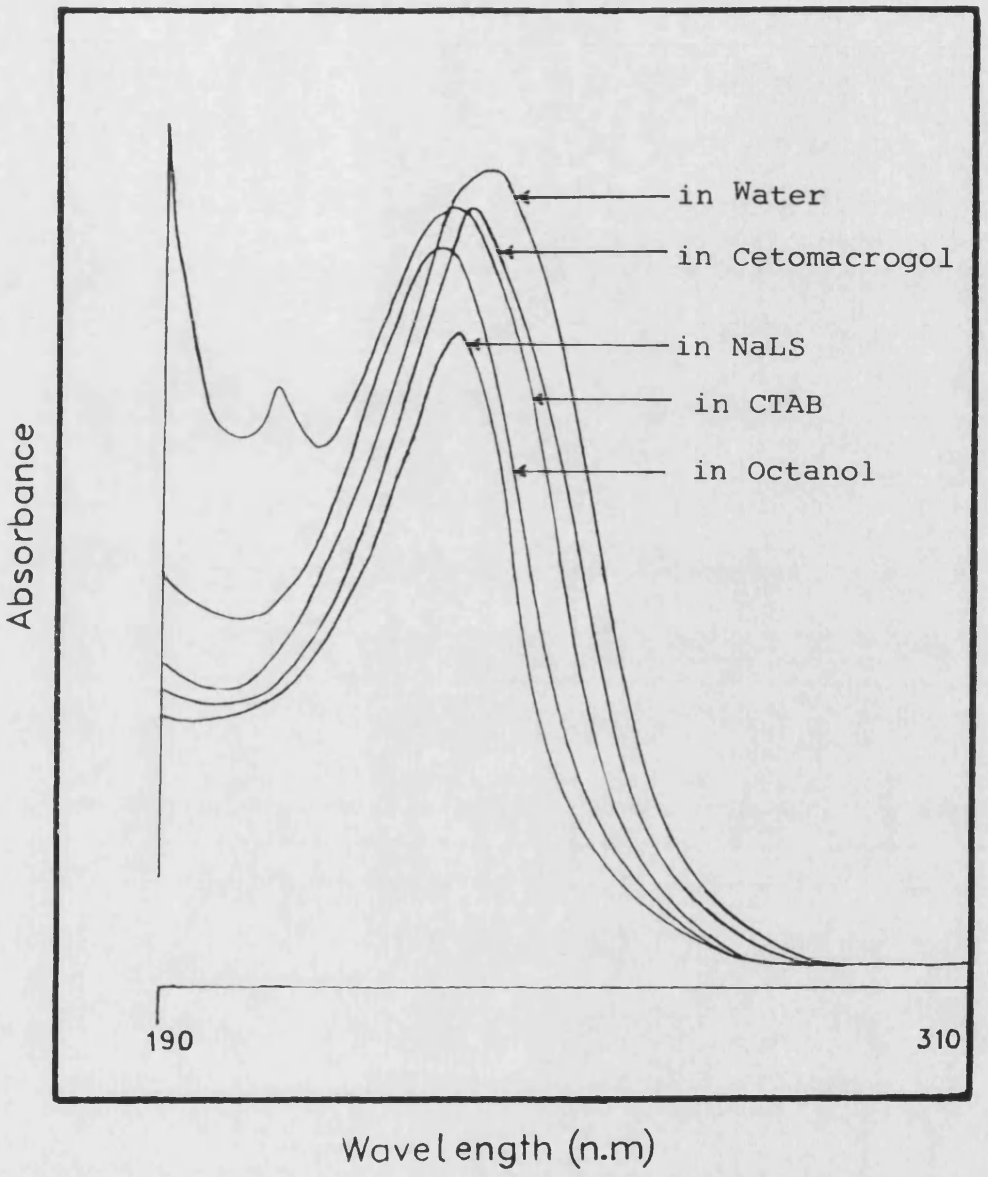


Fig.3.5.3 Absorbance Spectra of Hydrocortisone in Water, Aqueous Solutions of Surfactants and Octanol

Table 3.5.6 DATA OF λ_{MAX} OF HYDROCORTISONE IN DIFFERENT SURFACTANT SOLUTIONS COMPARED TO THAT IN WATER AND OCTANOL

SOLVENT	λ_{MAX} OF HYDROCORTISONE (nm.)
Water	245
1×10^{-3}M Cetomacrogol 1000	244
4×10^{-2}M NaLS	242
1×10^{-2}M CTAB	240
Octanol	238

3.5.4 Effect of CTAB, NaLS and Cetomacrogol 1000 on the Sensitivity of Hydrocortisone and Hydrocortisone Phosphate to the Hydroxyl Radical

Using the stock solutions of the three surfactants, the same series of concentrations of each surfactant containing 5.517×10^{-4}M hydrocortisone or 5.549×10^{-4}M hydrocortisone phosphate respectively were prepared. All the solutions were saturated with N_2O gas before irradiation. 2 ml samples of each solution were irradiated with different doses of radiation and analysed for the residual concentrations of the corticosteroid by reference to the unirradiated solution. After repeating the experiment, the mean reaction rates of the corticosteroids as well as the mean G^- values were calculated. Plots of G^- values against the molar concentration of surfactant are shown in figs. 3.5.4 and 3.5.5, from which it is evident that both corticosteroids are protected from the hydroxyl radical by the surfactants to different extents which can be placed in the order of NaLS > CTAB > Cetomacrogol 1000 in its protective effect.

3.5.5 Effect of CTAB, NaLS and Cetomacrogol 1000 on the Sensitivity of Hydrocortisone and Hydrocortisone Phosphate to Hydrogen Atom

The same series of concentrations of the three surfactants containing the same concentrations of corticosteroids were prepared and the pH of all solutions were adjusted to 1.27 using 4N HCl and then saturated with hydrogen gas. 2 ml samples of each solution were then irradiated with different doses of radiation, analysed for the residual concentrations of the corticosteroids by reference to the unirradiated solutions and the mean rate of reaction as well as the mean G^- value of each solution were calculated. Plots of G^- values against the molar concentration of surfactant are shown in figs. 3.5.4 and 3.5.5, from which it can be noted that the corticosteroids are protected from the hydrogen atom by the surfactants in the order NaLS $\gg$ CTAB $>$ Cetomacrogol 1000. Also, it can be noted that the corticosteroids are less protected from the hydrogen atom than the hydroxyl radical by the surfactants.

3.5.6 Effect of CTAB, NaLS and Cetomacrogol 1000 on the Sensitivity of Hydrocortisone and Hydrocortisone Phosphate to Hydrated Electron

The experiment was repeated using 2 molar solution of methanol as a solvent for preparing the solutions of the three surfactants and the pH of all the solutions were adjusted to 11.3 using N NaOH. 2 ml samples of each solution containing the respective corticosteroids were irradiated with different doses of radiation, analysed for the residual concentrations of corticosteroids by reference to the unirradiated solutions and the mean rate of reaction as well as the mean G^- value of each solution were calculated. Plots of G^- values against the molar concentration of surfactant are shown in figs. 3.5.4 and 3.5.5, from which it can be seen that the surfactants have very little protective effect on both corticosteroids compared to $\dot{O}H$ and $\dot{H}$ and this effect is apparently independent of the surfactant concentration.

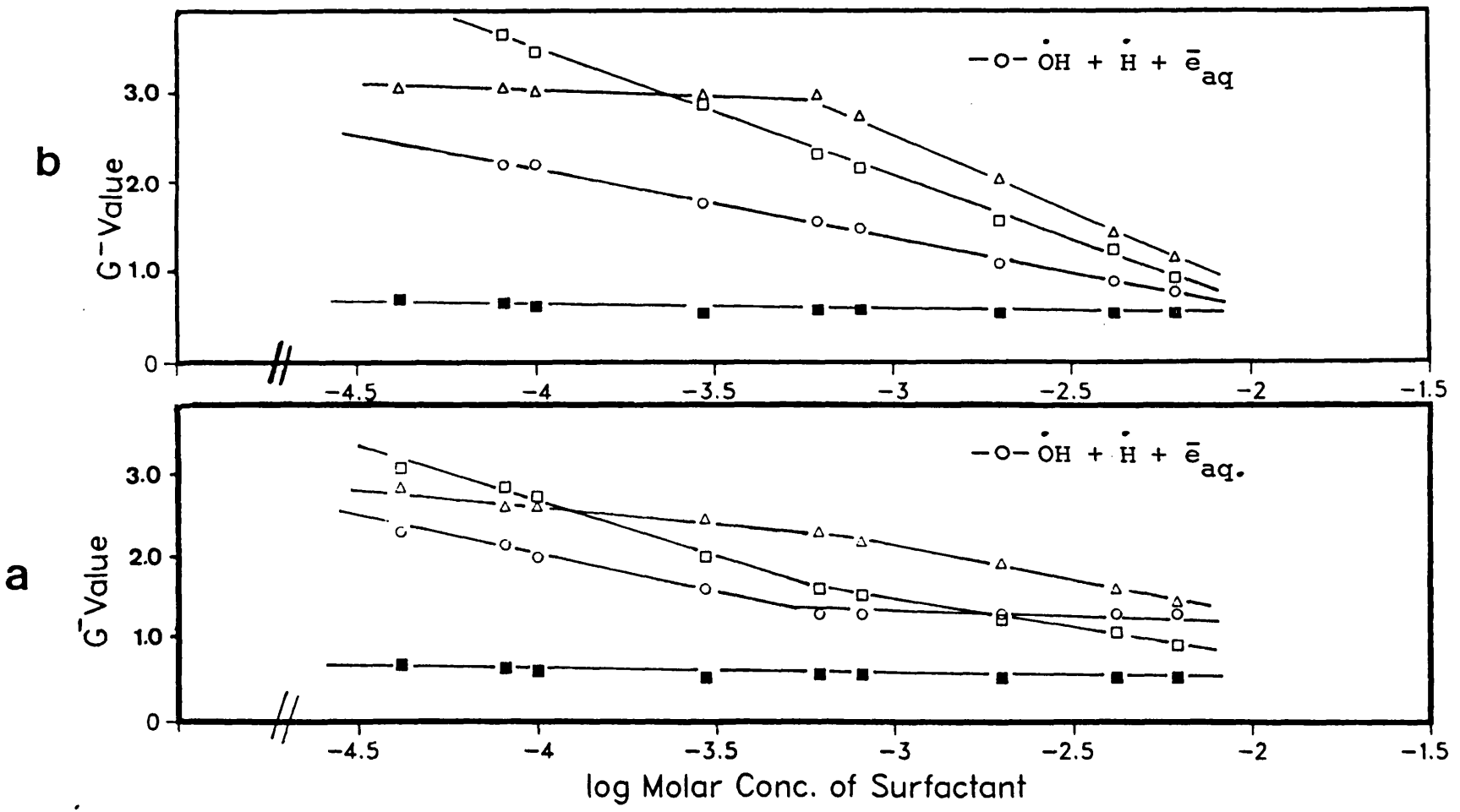


Fig. 3.5.4 Plots of G^- Values of Hydrocortisone (a) and Hydrocortisone Phosphate (b) Degradation by the Hydrogen Atom (△), Hydroxyl Radical (□) and Hydrated Electron (■) Against log Molar Concentration of CTAB

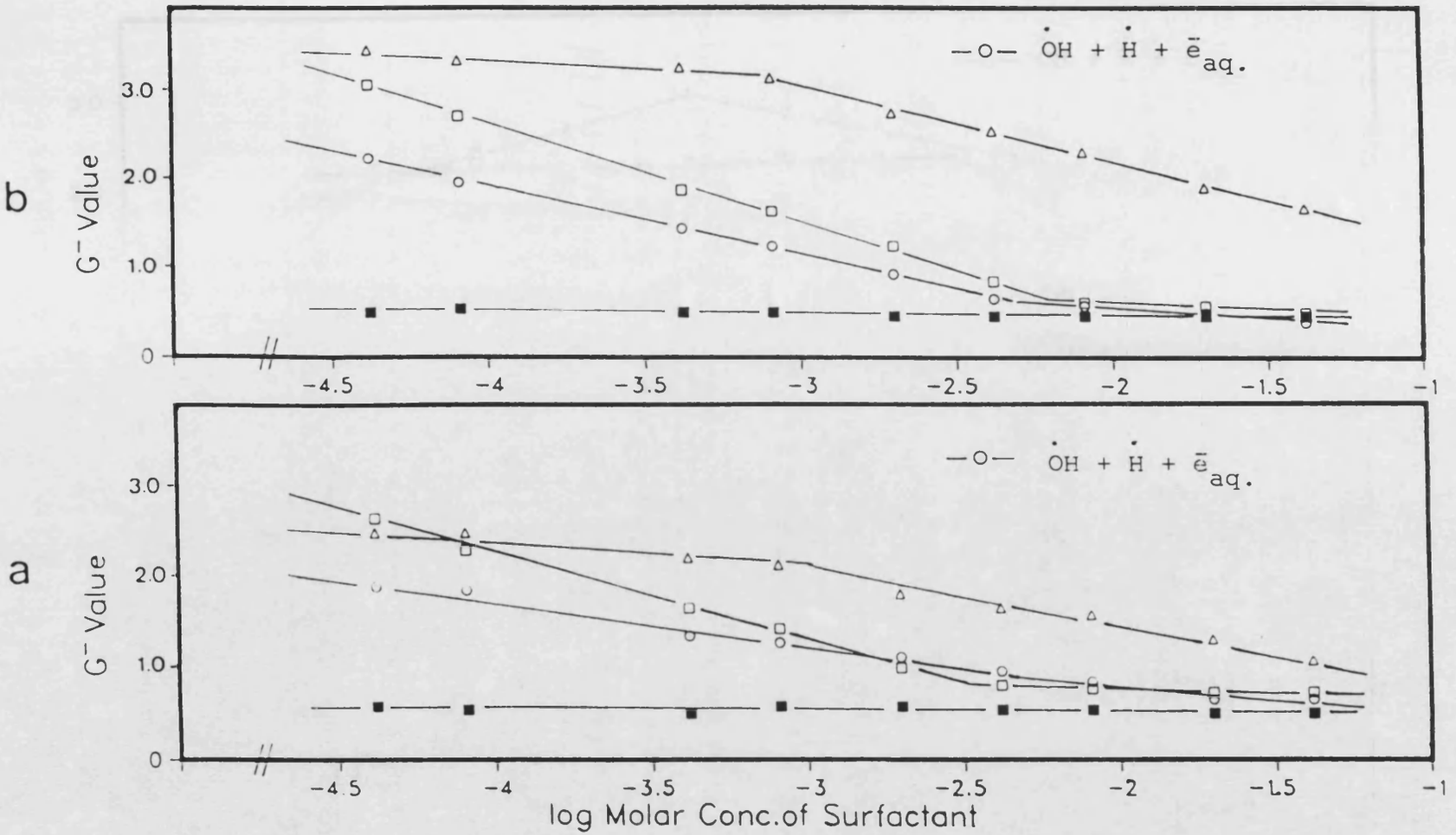


Fig 3.5.5 Plots of G^{-} Values of Hydrocortisone (a) and Hydrocortisone Phosphate (b) Degradation by the Hydrogen Atom (△), Hydroxyl Radical (□) and Hydrated Electron (■) Against Log Molar Concentration of NaLS

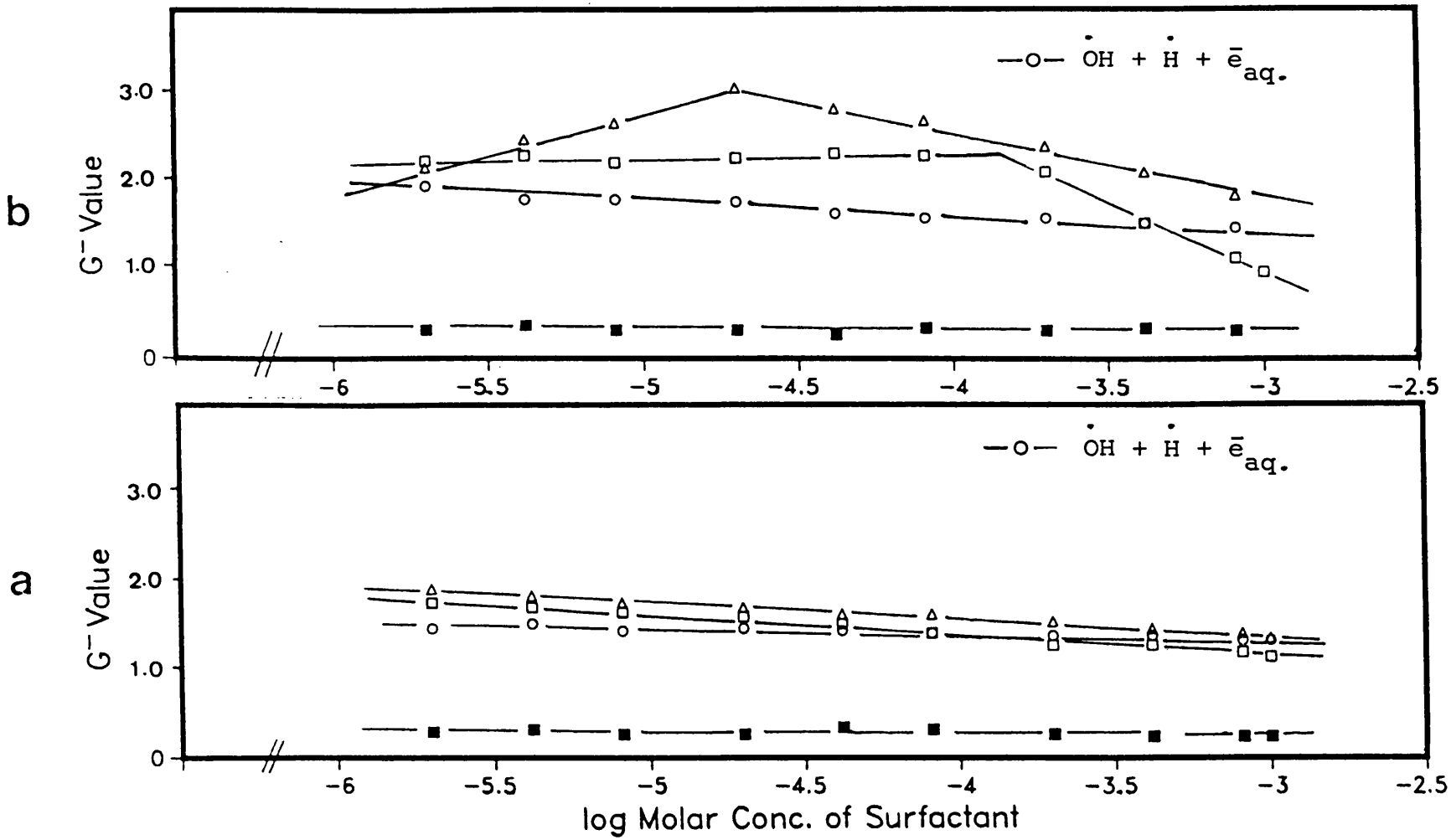


Fig. 3.5.6 Plots of G^- Values of Hydrocortisone (a) and Hydrocortisone Phosphate (b) Degradation by the Hydrogen Atom (△), Hydroxyl Radical (□) and Hydrated Electron (■) Against Log Molar Concentration of Cetomacrogol 1000

3.5.7 Determination of the CMC of the Surfactant Solutions

It is apparent from the results obtained by studying the effect of the surfactants on the sensitivity of hydrocortisone and hydrocortisone phosphate to γ-radiation that there are breaks in some of the plotted curves. To investigate whether these breaks were due to micellar formation or not, it was decided to determine the CMC of the respective surfactants in all solutions, under the specified irradiation conditions, and compare these CMC values with the concentrations at which breaks occurred in the curves. In addition, this comparison would help in the interpretation of the possible mechanisms of protection of the drug by the surfactants as well as the nature of the reaction between the drug and the surfactant and the latter with the radiolytic products of water.

The surface tension was measured by the standard Wilhelmy plate method according to the technique used by Shetewi[160] in the department, and was used for the determination of the CMC of the surfactants under the appropriate conditions.

Apparatus and Procedure:

The apparatus was constructed by modifying an Oertling balance. The principal components of the assembly are shown diagrammatically in fig. 3.5.6. A standard microscope cover glass, the plate, was attached to one of the pans with non-spun monofilament nylon thread with a platinum hook. This allowed for the exact balancing of the free plate and the nylon thread by counter-weights on the other balance pan. The plate, during determination, was housed in a special glass container which had a wide mouth to allow the plate to enter without touching the sides. During determination the container was covered with halved plastic petri dish which had a circular hole in the middle to allow for the free movement of the nylon thread. The container was mounted on a movable rack and pinion arrangement that could be raised or lowered very smoothly in a water bath kept at a constant temperature of $25^{o}C$. The vertical position of the plate can be checked by its reflected image in the solution which acted as a mirror.

The surface tension is given directly by the downward force acting upon the periphery of the wetted plate. The zero position of the plate was noted by the perfect balancing of the two pans of the balance. The balance beam was then clamped in this zero position. The solution was then gently raised until the plate just touched the surface of the solution. The balance beam was then released. As a result the plate sank into the solution. Counterweights were then placed on the other balance pan

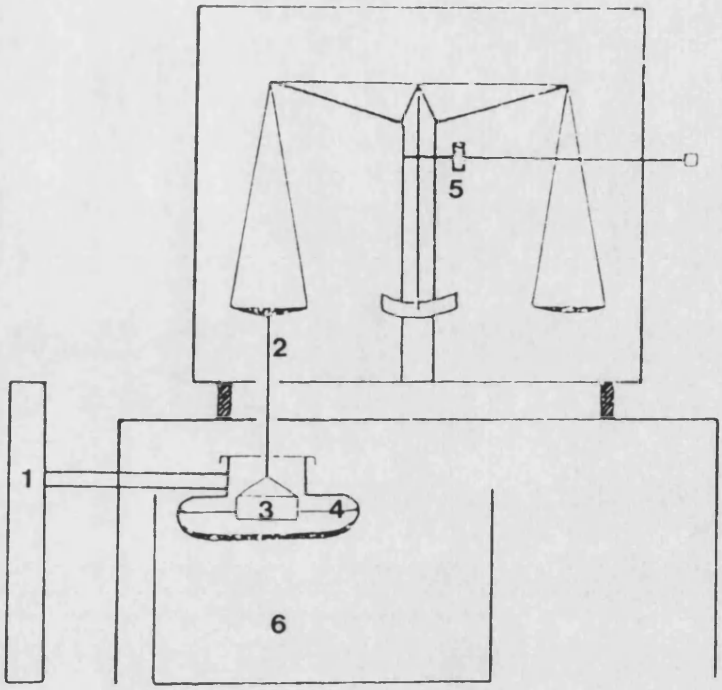


Fig. 3.5.7 Schematic Representation of the Wilhelmy Balance Arrangement for the Surface Tension Measurements

1. Rack and Pinion
2. Non-Spun Nylon Thread
3. Glass Cover Slip
4. Special Glass Flask with Cover
5. Chain Weight Arrangement
6. Water Bath

until the zero position was restored. The final balancing was made by using a chain weight attached to a circular scale mounted on the column of the balance. This enabled the accurate addition of 1 mg at a time. The final total weight was recorded. This was repeated three times for each determination and the average of the three readings was taken as the weight necessary to bring the plate to zero position.

The surface tension was then calculated from the following relationship:

$$\gamma = \frac{wg}{2(L + t)}$$

γ = Surface tension in dyne cm^{-1}

w = The weight, in grammes, necessary to bring the plate to the zero position.

g = The acceleration of gravity (981 cm sec^{-2})

L = The width of the plate in cm.

t = The thickness of the plate in cm.

L and t were determined using a travelling microscope.

Method:

The CMC of CTAB, NaLS and Cetomacrogol 1000 were determined through the surface tension measurements of a series of solutions of different concentrations of each surfactant and plotting these surface tension measurements against log molar concentration. Then these solutions were subjected to irradiation upto 3K.Grays and their CMC were again determined. The CMC of the three surfactants were determined in the following solutions:

1. In the presence of hydrocortisone before and after irradiation.
2. In the presence of hydrocortisone and $\dot{OH}$.
3. In the presence of hydrocortisone and $\dot{H}$.
4. In the presence of hydrocortisone and $\bar{e}$ aq.
5. In the presence of hydrocortisone phosphate before and after irradiation.
6. In the presence of hydrocortisone phosphate and $\dot{OH}$.
7. In the presence of hydrocortisone phosphate and $\dot{H}$.
8. In the presence of hydrocortisone phosphate and $\bar{e}$ aq.

The CMC obtained from the break in the curves were compared with the breaks in the curves obtained from the study of the effect of surfactants on the sensitivity of hydrocortisone and hydrocortisone phosphate to γ-radiation. The data are presented in tables 3.5.7,3.5.8 and 3.5.9 from which it is evident that the CMC of the three surfactants are lowered by the presence of hydrocortisone or hydrocortisone phosphate. Also, it can be seen that the breaks noted in the curves, representing the sensitivity of corticosteroids to different radiolytic species of water, are either around the determined CMC or at a higher concentration of the surfactant.

Table 3.5.7 COMPARISON BETWEEN THE MEASURED CMC OF CTAB AND THE BREAKS NOTED IN THE SENSITIVITY CURVES OF HYDROCORTISONE (a) AND HYDROCORTISONE PHOSPHATE (b)

(a)

Contents of Surfactant Solution	Molar Concentration of CTAB	
	Measured CMC	Break in the Curve
Water	9.6×10^{-4}	
Hydrocortisone	6.9×10^{-4}	
Hydrocortisone + 3 Radiolytic Species of Water	6.02×10^{-4}	9.3×10^{-4}
Hydrocortisone + $\dot{OH}$	7.5×10^{-4}	6.16×10^{-4}
Hydrocortisone + $\dot{H}$	4.5×10^{-5}	9.3×10^{-4}
Hydrocortisone + $\bar{e}$ aq.	5.6×10^{-4}	no break

Table 3.5.7 (continued)

(b)

Contents of Surfactant Solution	Molar Concentration of CTAB	
	Measured CMC	Break in the Curve
Water	9.6×10^{-4}	
Hydrocortisone Phosphate	1.25×10^{-5}	
Hydrocortisone Phosphate + 3 Radiolytic Species of Water	1.14×10^{-5}	no break
Hydrocortisone . Phosphate + OH	1.65×10^{-5}	no break
Hydrocortisone . Phosphate + H	3.31×10^{-5}	6.16×10^{-4}
Hydrocortisone Phosphate + $\bar{e}$ aq	1.00×10^{-5}	no break

Table 3.5.8 COMPARISON BETWEEN THE MEASURED CMC OF NaLS AND THE BREAKS NOTED IN THE SENSITIVITY CURVES OF HYDROCORTISONE (a) AND HYDROCORTISONE PHOSPHATE (b)

(a)

Contents of Surfactant Solution	Molar Concentration of NaLS	
	Measured CMC	Break in the Curve
Water	8×10^{-3}	
Hydrocortisone	6.6×10^{-3}	
Hydrocortisone + 3 Radiolytic Species of Water	7.4×10^{-3}	5.2×10^{-3}
Hydrocortisone + $\dot{O}H$	3.3×10^{-3}	3.16×10^{-3}
Hydrocortisone + $\dot{H}$	1.09×10^{-3}	1.09×10^{-3}
Hydrocortisone + $\bar{e}$ aq	1.00×10^{-3}	no break

Table 3.5.8 (continued)

(b)

Contents of Surfactant Solution	Molar Concentration of NaLS	
	Measured CMC	Break in the Curve
Water	8×10^{-3}	
Hydrocortisone Phosphate	6.91×10^{-3}	
Hydrocortisone Phosphate + 3 Radiolytic Species of Water	7.9×10^{-3}	3.9×10^{-3}
Hydrocortisone . Phosphate + OH	6.6×10^{-3}	7.4×10^{-3}
Hydrocortisone . Phosphate + H	8.31×10^{-4}	8.31×10^{-4}
Hydrocortisone Phosphate + $\bar{e}$ aq	9.25×10^{-4}	no break

Table 3.5.9 COMPARISON BETWEEN THE MEASURED CMC OF CETOMACROGOL 1000 AND THE BREAKS NOTED IN THE SENSITIVITY CURVES OF HYDROCORTISONE (a) AND HYDROCORTISONE PHOSPHATE (b)

(a)

Contents of Surfactant Solution	Molar Concentration of Cetomacrogol 1000	
	Measured CMC	Break in the Curve
Water	6.60×10^{-5}	
Hydrocortisone	3.46×10^{-5}	
Hydrocortisone + 3 Radiolytic Species of Water	7.94×10^{-5}	no break
Hydrocortisone + $\dot{O}H$	4.36×10^{-5}	no break
Hydrocortisone + $\dot{H}$	3.80×10^{-5}	no break
Hydrocortisone + $\bar{e}$ aq	7.21×10^{-5}	no break

Table 3.5.9 (Continued)

(b)

Contents of Surfactant Solution	Molar Concentration of Cetomacrogol 1000	
	Measured CMC	Break in the Curve
Water	6.6×10^{-5}	
Hydrocortisone Phosphate	5.75×10^{-5}	
Hydrocortisone Phosphate + 3 Radiolytic Species of Water	3.16×10^{-5}	no break
Hydrocortisone . Phosphate + OH	2.08×10^{-5}	1.6×10^{-4}
Hydrocortisone . Phosphate + H	1.51×10^{-5}	1.9×10^{-5}
Hydrocortisone Phosphate + $\bar{e}$ aq	7.01×10^{-5}	no break

3.6.1 Effect of Gamma-Radiation on Hydrocortisone in a Formulated Cream

Hydrocortisone is presented in the form of a cream for topical use and is an official preparation in the B.P.C. 1973 as a 1% w/w cream which contains cetomacrogol emulsifying ointment as an emulsifier. Since it is evident from the previous experiments that cetomacrogol 1000 has a protective effect on hydrocortisone against the radiolytic products of water, it was decided to investigate the effect of gamma-radiation on hydrocortisone in a cream formulation containing cetomacrogol. The chosen formula of B.P.C. 1973 consists of:

Cetomacrogol emulsifying ointment	30g
Chlorocresol	0.1g
Hydrocortisone	1g
Freshly boiled and cooled water	68.9g

and cetomacrogol emulsifying ointment consists of:

White soft paraffin	500g
Cetomacrogol emulsifying wax	300g
Liquid paraffin	200g

N.B. Cetomacrogol emulsifying wax consists of:

Cetomacrogol 1000	200g
Cetostearyl alcohol	800g

Preparation of Hydrocortisone Cream:

0.5g of hydrocortisone was levigated with 3g of liquid paraffin in a porcelain dish. To the dish was added 4.5g of cetomacrogol emulsifying wax and 7.5g of white soft

paraffin and the whole mixture was melted by means of a gentle heat on a water bath with continuous stirring. The aqueous phase containing 0.05g chlorocresol was added to the oily phase and thoroughly mixed to form an emulsion, which was stirred until cold then it was adjusted to the final weight of 50g with freshly boiled and cooled purified water and remixed.

Analysis of Cream

The cream was divided into four quadrants on a tile and a 1g aliquot was taken from each of the two diagonal quadrants and placed in round-bottom centrifuge tubes. The four quadrants were remixed, divided into four quadrants and 1g aliquots were again taken from each of the two diagonal quadrants and placed in round-bottom centrifuge tubes. These aliquots were extracted by adding 5 ml of methanol to each centrifuge tube which were then shaken for 1 hour. 4 ml of the methanol extracts were removed and placed in 25 ml volumetric flasks and the cream samples were subjected to further multiple extraction by shaking with 4 ml, 3 ml and 3 ml of methanol. The respective extracts were combined in the volumetric flasks and the combined extract volumes were then made up to 25 ml with methanol. 1 ml of each sample was mixed with 1 ml of methanolic solution of hydrocortisone acetate as an internal standard in a 10 ml volumetric flask and the volume made up to 10 ml with methanol. Three 20 µl aliquots of each of these solutions were then assayed by the standard HPLC method

using acetonitrile : water (40 : 60) as the mobile phase at a flow rate of 1.2 ml/minute and by reference to the mean peak height ratios of three 20 µl injections of a standard solution of hydrocortisone in methanol.

The percentages of the original concentration of hydrocortisone extracted were calculated and are presented in table 3.6.1.

On calculating the coefficient of variation for the extraction of hydrocortisone from the cream base, it was found to be 4.69% which was considered to be a high value. Therefore, for determining suitable reproducibility of extraction of the drug, it was decided to apply another method for extracting the corticosteroid from the cream.

Ultrasonic Extraction:

From the same cream 1g samples were taken by the same technique and placed in 25 ml stoppered conical flasks. The samples were extracted by adding 10 ml of methanol to each flask and placed in an ultrasonic bath for 30 minutes. 8 ml of the methanol extracts were removed, placed in 25 ml volumetric flasks and the samples were further extracted by multiple extraction using 8 ml, 5 ml and 5 ml of methanol. The combined extract volumes in each volumetric flask were made up to 25 ml with methanol and assayed as previously to determine the percentages of hydrocortisone which could be extracted. The results are presented in table 3.6.2 which shows a coefficient of variation of 0.612%. As this value is considerably lower

Table 3.6.1 DATA SHOWING PERCENTAGE EXTRACTION OF HYDROCORTISONE FROM 1% W/W CREAM

SAMPLE NUMBER	PERCENTAGE OF ORIGINAL CONCENTRATION OF HYDROCORTISONE EXTRACTED
1	95.22
2	95.22
3	87.25
4	95.22
5	86.87
6	87.63
7	95.97
8	96.35
Standard Deviation	4.34
Coefficient of Variation %	4.69

Table 3.6.2 DATA SHOWING PERCENTAGE EXTRACTION OF HYDROCORTISONE FROM 1% W/W CREAM USING ULTRASONIC BATH

SAMPLE NUMBER	PERCENTAGE OF ORIGINAL CONCENTRATION OF HYDROCORTISONE EXTRACTED
1	95.66
2	95.30
3	95.66
4	96.75
5	97.11
6	96.02
7	95.30
8	95.66
The mean	95.93
Standard Deviation	0.587
Coefficient of Variation %	0.612

than the previous extraction process, it would suggest that a reproducible extraction from samples of the formulated cream has been achieved, and an acceptable uniformity of dispersion of the drug in the base has been produced. Therefore, radiation experiments were carried out on hydrocortisone creams with and without chlorocresol in the formula to ascertain the effect of the antimicrobial agent on the radiation sensitivity of the corticosteroid.

Method:

Using Cobalt-60 source, the dose rate absorbed by the vessels specified for irradiating ointments and creams was first determined by irradiating 50 ml of freshly prepared dosimetry solution for 10, 20, 30, 40 and 60 minutes and the mean dose rate was found to be 7.48 Gy/min.

Two creams containing 1% w/w hydrocortisone were prepared. The first contained the specified amount of chlorocresol while the second contained no chlorocresol. 50g of each formula were placed in a large irradiation vessel and exposed to ionising radiation up to 27.15 K.Gray, which is beyond the recommended sterilisation dose by B.P. (25 K.Gray). Duplicate samples, 1g each, were taken at appropriate dose intervals and placed in 25 ml stoppered conical flasks. All the samples were subjected to multiple extraction using the ultrasonic bath as before and assayed for the residual concentration of hydrocortisone by reference to the unirradiated samples extracted side by side each time.

The data obtained are presented in table 3.6.3 which show that chlorocresol has a stabilising effect on

hydrocortisone in the formula, but even in the absence of chlorocresol it is evident that the presence of cetomacrogol emulsifying wax and possibly the other ingredients, have a considerable protective effect against radiation.

Table 3.6.3 THE EFFECT OF CHLOROCRESOL ON THE SENSITIVITY OF HYDROCORTISONE IN CREAM TO IONISING RADIATION

Dose of Radiation (K.Gy)	Percentage Residual Concentration of Hydrocortisone	
	With Chlorocresol	Without Chlorocresol
0	100	100
3.36	98.71	99.01
7.39	99.11	97.15
11.25	98.25	96.89
14.21	97.31	98.17
18.01	98.22	97.77
20.12	99.16	95.98
23.17	100.02	96.95
25.31	97.77	97.13
27.15	97.13	95.83

3.6.2 Effect of Gamma-Radiation on Hydrocortisone in a Formulated Ointment

Hydrocortisone is presented in the form of an ointment for topical use as an official preparation in the B.P.[1] consisting of a mixture of liquid paraffin and white soft paraffin. In the Nordic P., hydrocortisone is formulated in an ointment base containing 0.75% w/w propylene glycol, 5% w/w cetyl alcohol, 19.25% w/w liquid paraffin and 75% w/w soft paraffin. Hayes[6] has reported that the presence of 2.5 - 5% w/w of propylene glycol in a white soft paraffin ointment base caused degradation of beclomethasone dipropionate up to 50% by ionising radiation at serilisation dose. Therefore it was decided to compare the sensitivity of both the B.P. and the Nord. P. formulae to gamma-radiation, investigating the effect of each ingredient in the Nord. P. formula on the sensitivity of the drug.

Method: Four ointments containing 1% w/w of hydrocortisone were prepared as follows:

1. 50g of 1% w/w hydrocortisone ointment were prepared by the levigation of 0.5g hydrocortisone in 10g liquid paraffin in a porcelain dish and mixed thoroughly by means of gentle heat with 39.5g of white soft paraffin and then the ointment was allowed to cool with constant mixing.

2. 50g of 1% w/w hydrocortisone ointment were prepared by levigating 0.5g of hydrocortisone in 9.62g liquid paraffin and 0.375g propylene glycol in a porcelain

dish. The levigated mixture was thoroughly mixed with 39.5g of white soft paraffin by gentle heat and then allowed to cool with constant mixing.

3. 50g of 1% w/w hydrocortisone ointment were prepared by levigating 0.5g of hydrocortisone in 10g liquid paraffin in a porcelain dish. 2.5g of cetyl alcohol, 37g of white soft paraffin were added and melted by means of gentle heat and the mixture was then allowed to cool with constant mixing.

4. 50g of 1% w/w hydrocortisone ointment were prepared by levigating 0.5g of hydrocortisone in 9.63g liquid paraffin and 0.375g of propylene glycol in a porcelain dish. 2.5g cetyl alcohol, 37g of white soft paraffin were added and melted by means of gentle heat and the mixture was allowed to cool with constant mixing.

30g of each ointment were placed in a large irradiation vessel and exposed to ionising radiation up to 27.15 K.Gray. Duplicate samples, 1g each, were taken at appropriate dose intervals and placed in 25 ml stoppered conical flasks. All the samples were gently heated in a water bath to ensure efficient extraction. The samples were subjected to multiple extraction as before and assayed for the residual concentration of hydrocortisone by reference to the unirradiated samples extracted side by side each time. The data obtained are presented in table 3.6.4 which show that the percentages of residual concentration of hydrocortisone in the four formulae comply with the

specification in the B.P. although the formula containing propylene glycol without cetyl alcohol is the least stable to radiation.

Further investigation of the effect of propylene glycol content in the ointment base on the sensitivity of hydrocortisone to radiation was carried out by repeating the experiment to include the percentage of propylene glycol 2%, 5% and 10% w/w in the formulation, displacing an equal amount of liquid paraffin to maintain the consistency of the ointments. The ointments were subjected to γ-radiation up to 27.15 K.Gray, extracted and assayed as before. The obtained results are shown in fig. 3.6.1 where the percentage residual concentration of hydrocortisone is plotted against dose of radiation for formulae containing different percentages of propylene glycol.

From fig. 3.6.1, it is clear that propylene glycol increases the sensitivity of hydrocortisone in an ointment base to radiation.

Table 3.6.4 THE SENSITIVITY OF 1% W/W HYDROCORTISONE IN OINTMENT BASES TO IONISING RADIATION

Dose of Radiation (K.Gy.)	Percentage Residual Concentration of Hydrocortisone			
	B.P. Formula	Nordic P. Formula		
		Complete	Excluding Propylene Glycol	Excluding Cetyl Alcohol
0	100	100	100	100
3.36	99.18	99.72	98.91	99.82
7.39	98.81	101.18	99.15	99.11
11.25	99.01	99.63	101.11	98.07
14.21	97.15	98.36	98.16	98.61
18.01	97.87	98.71	98.81	97.12
20.12	97.17	97.91	97.33	96.88
23.17	96.19	97.12	97.09	95.28
25.31	95.92	96.53	96.81	94.22
27.15	95.16	96.41	96.23	93.77

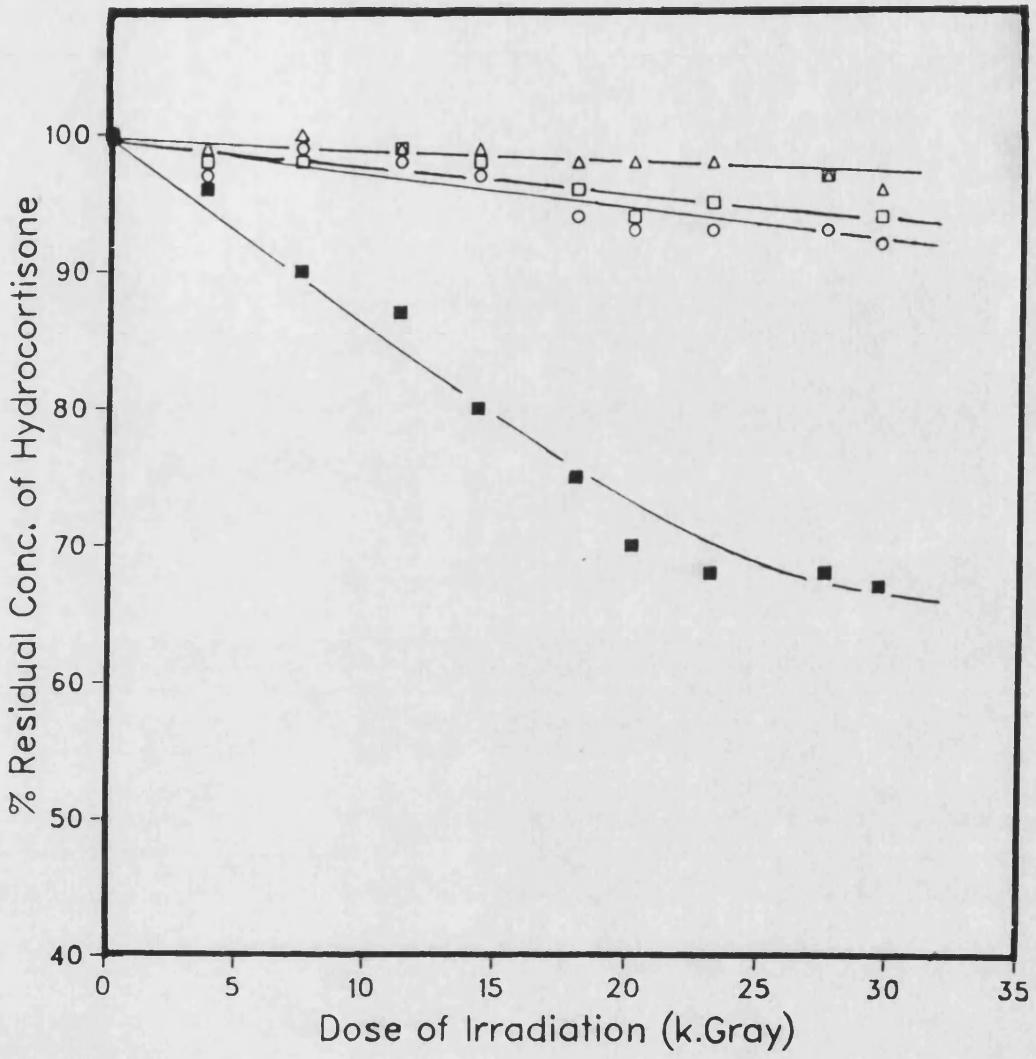


Fig. 3.6.1 The Effect of the Percentage Propylene Glycol on the Sensitivity of Hydrocortisone to Ionising Radiation in Nordic P. Formula Ointment

△ Nordic P. Formula

□ 2% W/W Propylene Glycol

○ 5% W/W Propylene Glycol

■ 10% W/W Propylene Glycol

3.7. Studies on the Degradation Products of Gamma-Irradiated Hydrocortisone, Hydrocortisone Acetate and Hydrocortisone Phosphate in Water and Propylene Glycol

During the quantitative HPLC determinations of the effect of gamma-radiation on hydrocortisone, hydrocortisone acetate and hydrocortisone phosphate in either water or in propylene glycol it became evident from the typical chromatograms, obtained, that the peaks of the three corticosteroids decreased in height and peaks of degradation products appeared. To investigate these degradation products resulting from the irradiation of the three corticosteroids either in aqueous or propylene glycol solutions, it was considered necessary to try and find a single HPLC system for their separation. The problem in trying to achieve such an ideal system was the apparent difference in polarity between hydrocortisone phosphate - the most polar - and hydrocortisone acetate - the least polar. In addition it has to be remembered that not all the degradation products, produced, would necessarily absorb in the u.v. and therefore may not be detected on the u.v. variable detector. Therefore an attempt to use a single HPLC system to study the separated degradation products of all three corticosteroids was carried out.

3.7.1 HPLC Separation of the Degradation Products of Hydrocortisone, Hydrocortisone Acetate and Hydrocortisone Phosphate After Gamma-Irradiation

The following solutions were prepared:

1. Hydrocortisone in propylene glycol 2mg/ml
2. Hydrocortisone in water 0.2mg/ml
3. Hydrocortisone phosphate in propylene glycol 2mg/ml
4. Hydrocortisone phosphate in water 2mg/ml
5. Hydrocortisone acetate in propylene glycol 2mg/ml

2 ml samples of each solution were irradiated up to 3 K.Grays for aqueous solutions and 20 K.Grays for the propylene glycol solutions. After subjecting the solutions to the respective doses of radiation, 20 µl aliquots of each solution were injected onto the reverse phase HPLC column using a mobile phase consisting of 50 : 50 methanol, 0.09M KH_2PO_4 at a flow rate of 1 ml/minute and chart speed of 1 cm/minute. The peaks were detected at a wavelength of 248 nm. and an absorbance range of 0.05 AUFS at ambient temperature.

The data for the capacity ratio values obtained for the peaks of possible degradation products are presented in table 3.7.1 and the HPLC traces are shown in figs. 3.7.1., 3.7.2 and 3.7.3. It is evident from these figures that some peaks of degradation products have been detected but they are relatively small compared to the parent corticosteroids. It was decided therefore to try to separate these products using thin layer chromatography in order to see if better separation could be achieved and possibly a greater number of degradation products separated.

Table 3.7.1 DATA FOR THE CAPACITY RATIO (K`) VALUES FOR OBSERVED PEAKS OBTAINED AS POSSIBLE DEGRADATION PRODUCTS FROM IRRADIATED SOLUTIONS OF HYDROCORTISONE, HYDROCORTISONE PHOSPHATE AND HYDROCORTISONE ACETATE IN WATER AND PROPYLENE GLYCOL

Capacity Factor (K`)	Hydrocortisone		Hydrocortisone Phosphate		Hydrocortisone Acetate
	Water	Propylene Glycol	Water	Propylene Glycol	Propylene Glycol
0.83	-	-	+	+	-
1.66	-	+	-	-	-
2.35	-	-	Hydrocortisone Phosphate	Hydrocortisone Phosphate	-
3.58	-	-	-	-	+
4.00	+	-	-	-	-
4.41	Hydrocortisone	Hydrocortisone	-	-	-
7.08	+	+	+	+	+
7.66	+	+	+	+	-
8.66	-	-	-	-	Hydrocortisone Acetate

$K` = \frac{R_t - R_o}{R_o}$ $K`$ = Capacity Ratio R_t = Retention time of the Compound

R_o = Retention time of the Solvent

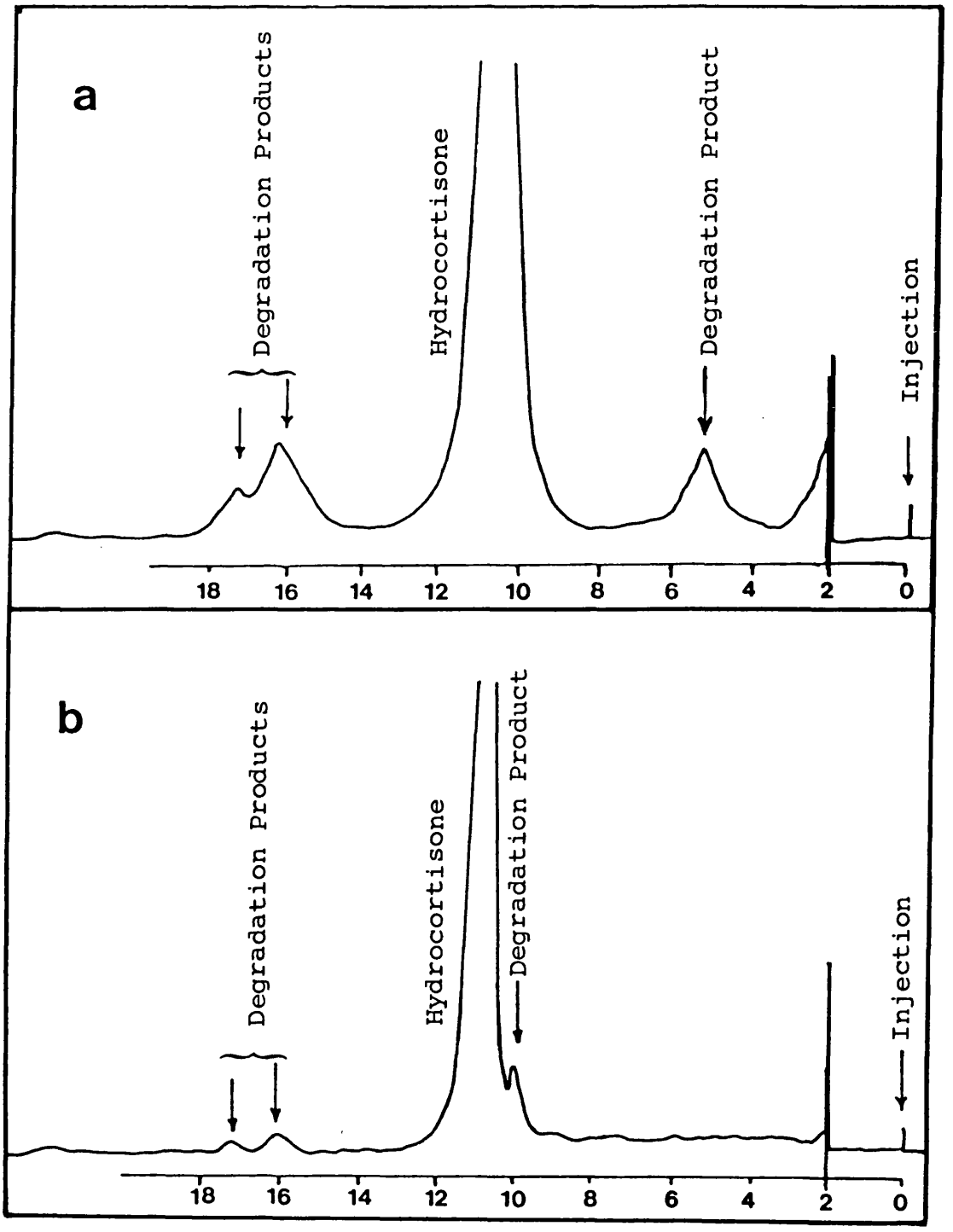


Fig. 3.7.1 HPLC Trace for Hydrocortisone and its Degradation Products After 20 K.Gray in Propylene Glycol (a) and After 3 K.Gray in Water (b)

Chromatographic Conditions:

Temperature: Ambient Flow Rate: 1 ml/min.

Chart Speed: 1 cm/min.

Absorbance Range: 0.05 AUFS at 248 nm.

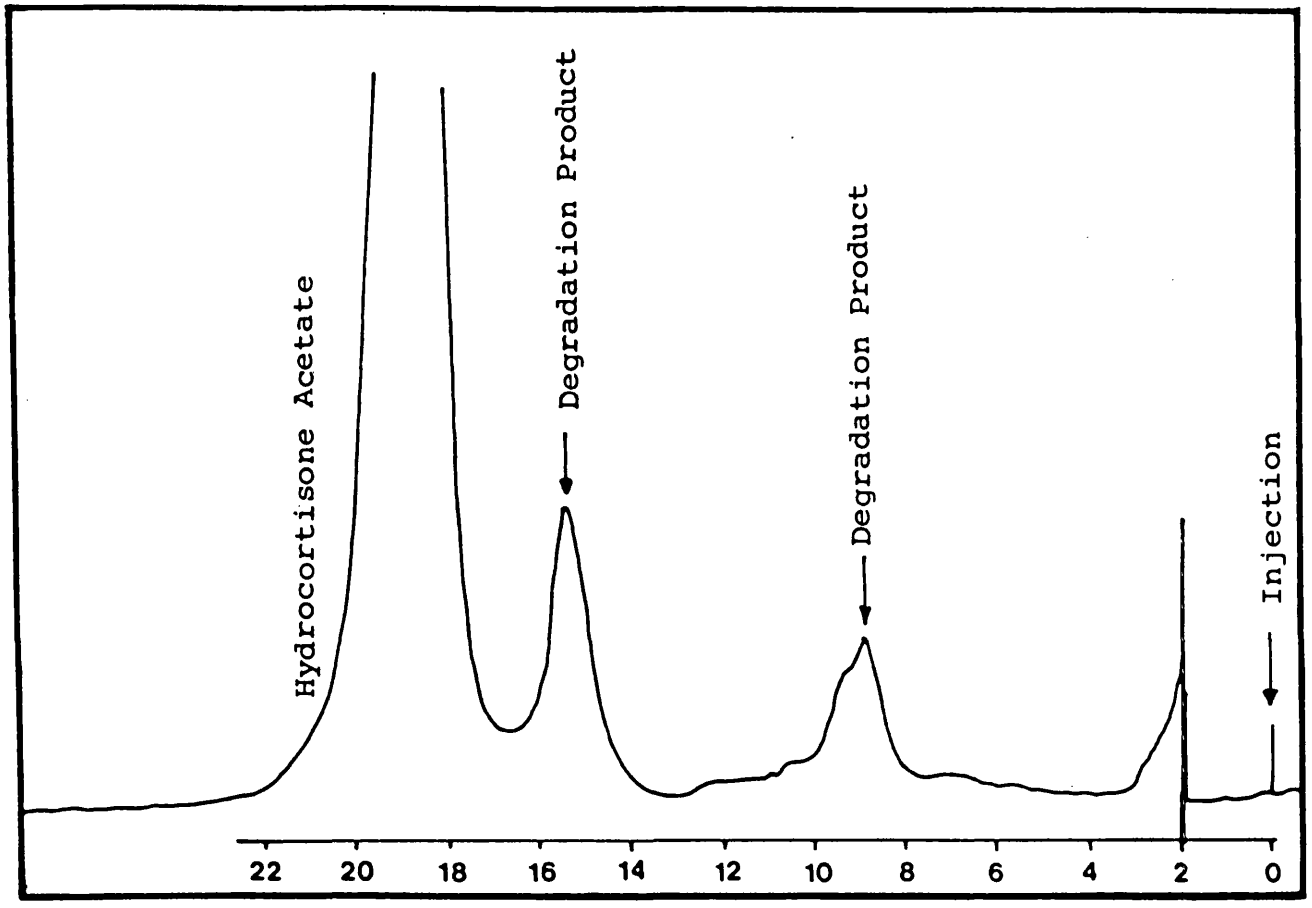


Fig. 3.7.2 HPLC Trace for Hydrocortisone Acetate and its Degradation Products After 20 K.Gray in Propylene Glycol

Chromatographic Conditions: Temperature: Ambient Flow Rate: 1 ml/min.
Chart Speed: 1 cm/min. Absorbance Range: 0.05 AUFS at 248 nm.

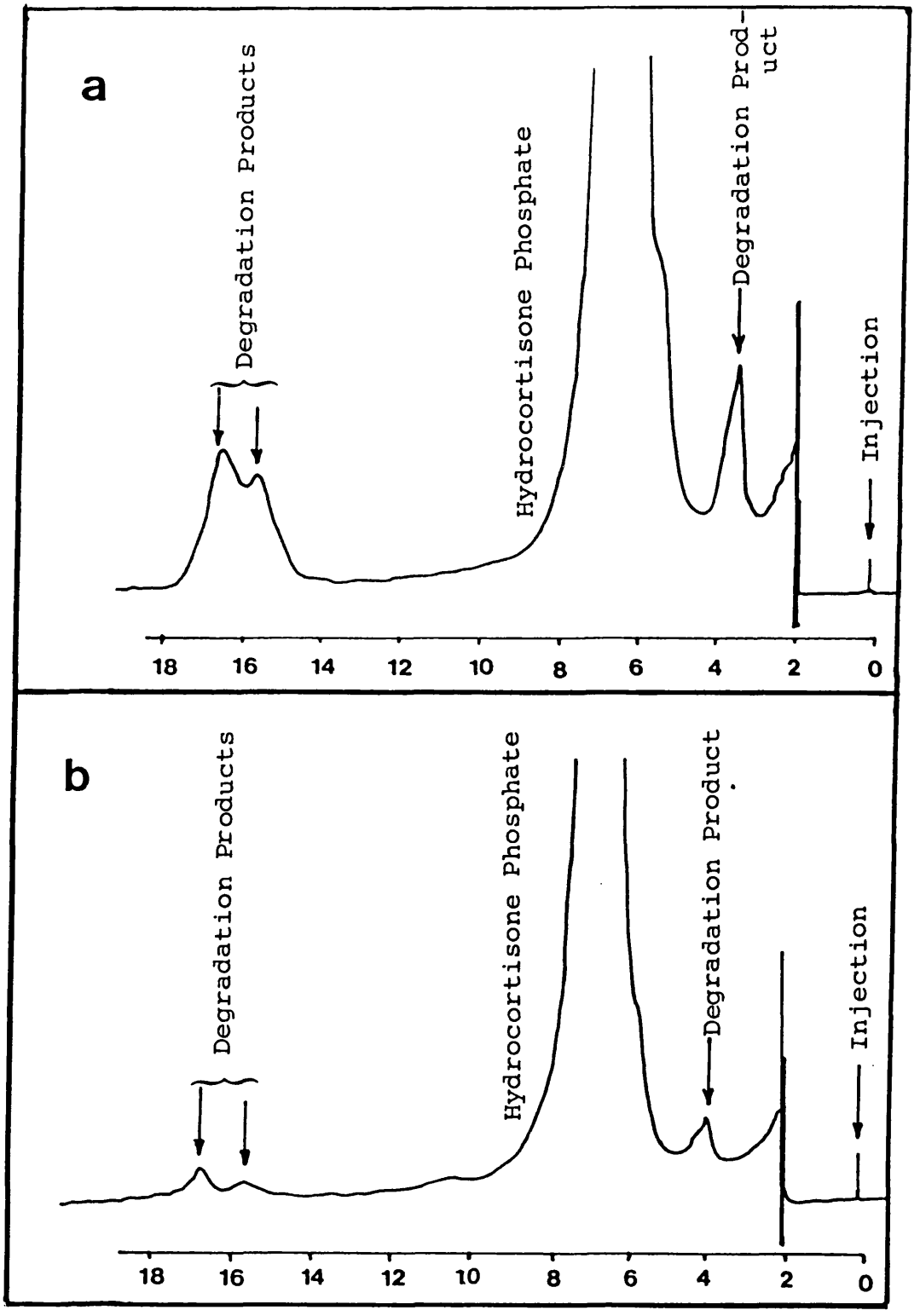


Fig. 3.7.3 HPLC Trace for Hydrocortisone Phosphate and its Degradation Products After 20 K.Gray in Propylene Glycol (a) and after 3 K.Gray in Water (b)

Chromatographic Conditions:

Temperature: Ambient Flow Rate: 1 ml/min.

Chart Speed: 1 cm/min.

Absorbance Range: 0.05 AUFS at 248 nm.

3.7.2 Thin Layer Chromatographic Separation of the Degradation Products of Hydrocortisone, Hydrocortisone Phosphate and Hydrocortisone Acetate After Irradiation

The separation of corticosteroids by TLC is well documented[6,124]. Different systems have been successfully applied for separation and detection of these corticosteroids even in the presence of their degradation products[124]. In the British Pharmacopoeia[1] silica gel G chromatoplates are used as the stationary phase and a mixture of 1,2 dichloroethane, methanol and water (95 : 5 : 0.2) as the eluting mobile phase. The reducing steroids, containing a $-COCH_2OH$ side chain, separated on these plates, can be detected by spraying with alkaline solution of 0.2% w/v blue tetrazolium in water or methanol. 16 hydroxy-17 ketosteroids and 2-hydroxy-3 ketosteroids also give +ve spots with the tetrazolium blue reagent[143]. To detect also those steroids or the degradation products not containing the reducing side chain, it was decided to use silica gel GF containing a u.v. phosphor indicator which highlights any steroid containing a carbonyl-double bond conjugation under a u.v. lamp at 254 nm. and 366 nm.

Method:

Solutions of hydrocortisone, hydrocortisone phosphate and hydrocortisone acetate (2mg/ml) in propylene glycol were prepared. 2 ml samples of each solution were irradiated for 20 K.Gray. Using glass capillary tubes, about 10 µl of the unirradiated and irradiated solutions were spotted

onto 0.2mm plastic sheets of silica gel along a base line 30 mm from the bottom of the sheets. The sheets were then developed in a chromatography tank which had been previously equilibrated for 1 hour with the eluting solvent consisting of 1,2 dichloromethane : Dioxane : H_2O (120 : 30 : 50). The eluting solvent was allowed to run to 135 mm from the base line which took approximately 2 hours, after which the sheets were removed and air dried.

Duplicate chromatograms for each solution were processed. One was subjected to u.v. light at 254 nm. and 366 nm., while the other was sprayed with alkaline tetrazolium blue reagent.

The data indicating the possible degradation products and their corresponding R_f* values obtained from the chromatograms, viewed under the u.v. light and sprayed with the alkaline tetrazolium blue reagent are presented in table 3.7.2.

$$^{*}R_f = \frac{\text{The distance from the base line to centre of spot zone}}{\text{The distance from the base line to solvent front}}$$

The chromatogram viewed under the u.v. light is shown in fig. 3.7.3.

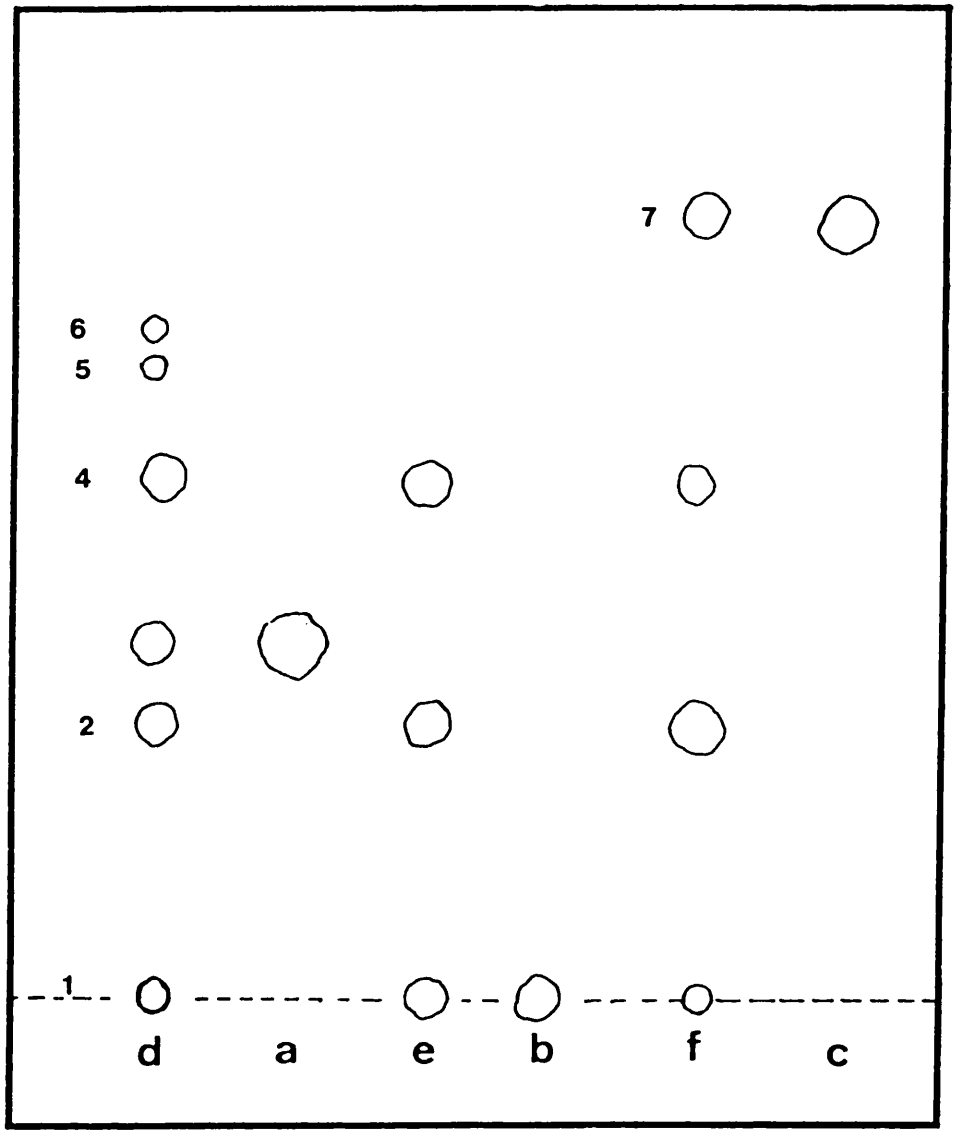


Fig. 3.7.3 T.L.C. Chromatogram for Irradiated and Unirradiated Hydrocortisone, Hydrocortisone Phosphate and Hydrocortisone Acetate in Propylene Glycol Detected under u.v. Light at 254 nm.

(a) Unirradiated Hydrocortisone

(b) Unirradiated Hydrocortisone Phosphate

(c) Unirradiated Hydrocortisone Acetate

(d) Hydrocortisone Irradiated for 20K.Gray in Propylene Glycol

(e) Hydrocortisone Phosphate Irradiated for 20K.Gray in Propylene Glycol

(f) Hydrocortisone Acetate Irradiated for 20K.Gray in Propylene Glycol

Table 3.7.2 DATA SHOWING R_f VALUES FOR HYDROCORTISONE, HYDROCORTISONE PHOSPHATE AND HYDROCORTISONE ACETATE AND THE POSSIBLE DEGRADATION PRODUCTS SEPARATED BY STRAIGHT PHASE TLC

Observed Spots	Detection by Tetrazolium Blue	R_f Value		
		Hydrocortisone +ve	Hydrocortisone Phosphate -ve	Hydrocortisone Acetate +ve
1	+ve	0	0	0
2	+ve	0.39	0.37	0.37
3	+ve	0.51		
4	+ve	0.64	0.67	0.65
5	-ve	0.73		
6	-ve	0.80		
7	+ve			0.85
Observed R_f Value for Unirradiated Corticosteroids		0.51	0	0.85

3.7.3 Reverse-Phase Thin Layer Chromatographic Separation of the Degradation Products of Hydrocortisone, Hydrocortisone Phosphate and Hydrocortisone Acetate After Radiation

From the straight phase TLC separation, it is obvious that hydrocortisone phosphate is strongly adsorbed on the silica gel particles of the stationary phase because of its high polarity. Therefore, it was decided to use reverse-phase stationary phase which is similar to that of the HPLC column (but containing the u.v. indicator). This would help to achieve simultaneous separation of the three steroids with the same mobile phase on the same plate. The mobile phase used was the same as that used in the HPLC separation.

Method:

The same solutions were prepared and irradiated. Using glass capillary tubes, about 10 µl of the unirradiated and irradiated solutions were spotted on to 0.2 mm chromatoplates of reverse-phase silica gel along a base line 30 mm from the bottom of the plates. The plates were then developed in a chromatography tank which had been previously equilibrated for 1 hour with eluting solvent methanol : 0.09M KH_2PO_4 (55 : 45), The eluting solvent was allowed to run to 135 mm from the base line which took approximately 2.5 hours, after which the plates were removed and air dried.

Duplicate chromatograms for each solution were processed. One was subjected to u.v. light at 254 nm. and

366 nm., while the other was sprayed with alkaline tetrazolium blue reagent. The data, indicating the possible degradation products and their corresponding R_f values obtained from the chromatograms viewed under u.v. light and sprayed with tetrazolium blue reagent, are presented in table 3.7.3. One of the chromatograms obtained, is shown in fig. 3.7.4.

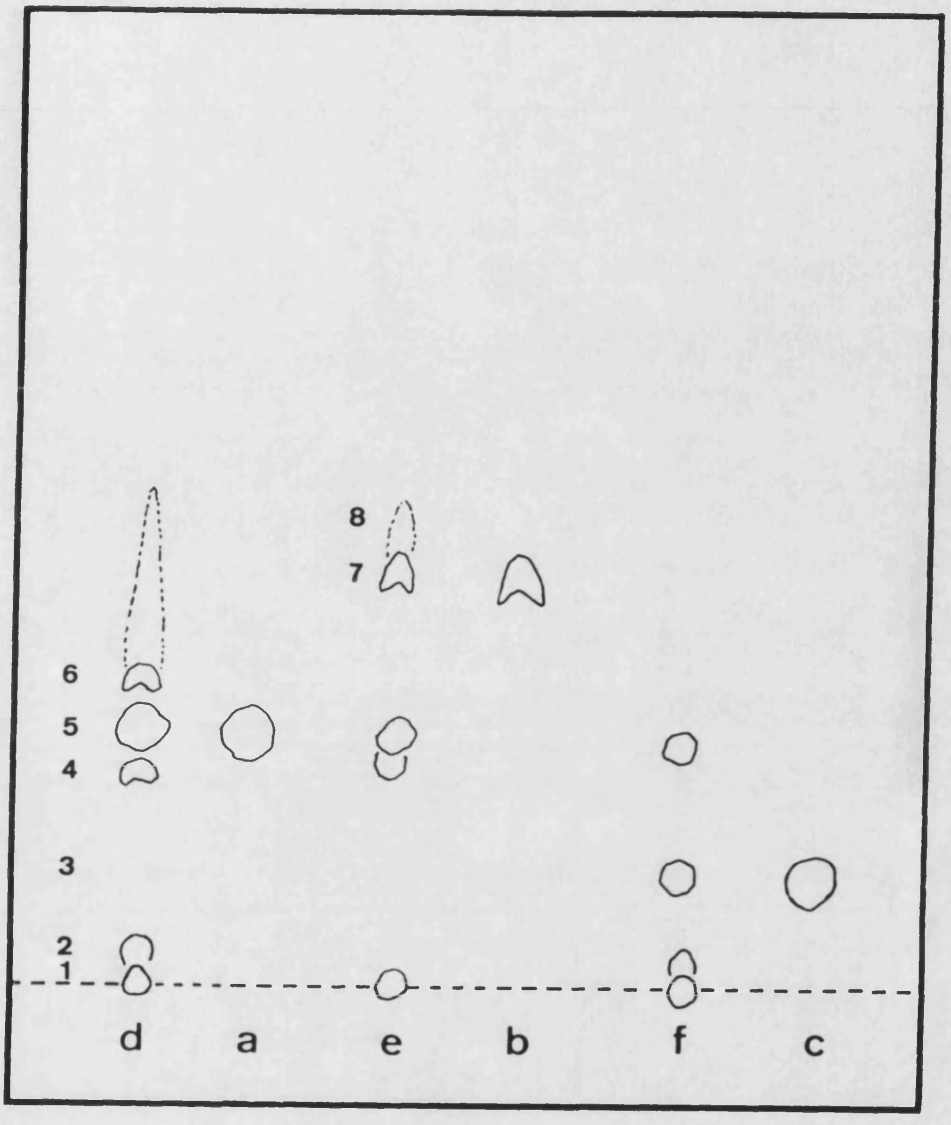


Fig. 3.7.4 Reverse-Phase T.L.C. Chromatogram for Irradiated and Unirradiated Hydrocortisone, Hydrocortisone Phosphate and Hydrocortisone Acetate in Propylene Glycol Detected Under u.v. Light at 254 nm.

(a) Unirradiated Hydrocortisone
(b) Unirradiated Hydrocortisone Phosphate
(c) Unirradiated Hydrocortisone Acetate
(d) Hydrocortisone irradiated for 20 K.Gray in Propylene Glycol
(e) Hydrocortisone Phosphate Irradiated for 20 K.Gray in Propylene Glycol
(f) Hydrocortisone Acetate irradiated for 20 K.Gray in Propylene Glycol

Table 3.7.3 DATA SHOWING R_f VALUES FOR HYDROCORTISONE, HYDROCORTISONE PHOSPHATE AND HYDROCORTISONE ACETATE AND THE POSSIBLE DEGRADATION PRODUCTS SEPARATED BY REVERSE-PHASE TLC

Observed Spots	Detection by Tetrazolium Blue	R_f Value		
		Hydrocortisone	Hydrocortisone Phosphate	Hydrocortisone Acetate
1	+ve	O (366nm)		O (366nm)
1	-ve		O (366nm)	
2	+ve	0.034	-	0.034
3	+ve	-	-	0.083 (Hc. Acetate)
4	+ve	0.104	0.105	-
5	+ve	0.166 (Hydrocortisone)	0.166	0.165
6	+ve	0.208 (366nm)	-	-
7	-ve	-	0.343 (Hc. Phosphate)	-
8	+ve	-	0.430 (366nm)	-

3.7.4 Separation of the Degradation Products of Hydrocortisone, Hydrocortisone Phosphate and Hydrocortisone Acetate on Preparative Thin Layer Chromatoplates

The previous TLC separations indicate that a reasonable separation of the degradation products of hydrocortisone, hydrocortisone phosphate and hydrocortisone acetate was achieved. It was decided therefore to use preparative straight phase TLC for the separation of more concentrated solutions and subsequently extract and concentrate the separated spots for analysis by HPLC to ascertain if these degradation products correspond to those previously highlighted by the standard HPLC traces.

Method:

Solutions of 10mg/ml of hydrocortisone, hydrocortisone phosphate and hydrocortisone acetate in propylene glycol were prepared. 2 ml samples of each solution were irradiated upto 40K.Gray. Using a micro-syringe 200 µl of each of the irradiated solutions were spotted as a continuous band on to 2 mm chromatoplates of silica gel GF along a base line 30 mm from the bottom of the plate. The chromatoplates were developed in chromatography tanks which had previously been equilibrated with eluting solvent of 1,2 dichloromethane : Dioxane : water (120 : 30 : 50). The eluting solvent was allowed to run to 150 mm from the base line after which the plates were removed and air dried. The plates were subjected to u.v.

light at 254 nm. and 366 nm. The bands detected under the u.v. light were marked as shown in fig. 3.7.5 and table 3.7.4 and each band was scraped off into a 100 ml stoppered conical flask. 25 ml of methanol was added to each flask and shaken for 5 minutes. The supernatant methanolic solution from each flask was filtered through filter paper into an evaporating dish. The extraction process in each flask was repeated twice using 25 ml methanol each time and again filtered through the same filter paper and collected in the evaporating dish. The methanolic extracts, obtained, were evaporated to dryness under vacuum at 40°C. Each residue was redissolved in 2 ml of methanol and 20 µl aliquots were injected on to the HPLC column using a mobile phase consisting of (50 : 50) methanol : 0.09M KH_2PO_4 at a flow rate of 1 ml/minute and chart speed of 0.2 cm/minute. The peaks were detected at a wavelength of 248 nm. and 366 nm. (for the band No. 6) and absorbance range of 0.05 AUFS at ambient temperature. Typical HPLC traces resulting from each band separated by TLC are illustrated in figs. 3.7.6, 3.7.7 and 3.7.8.

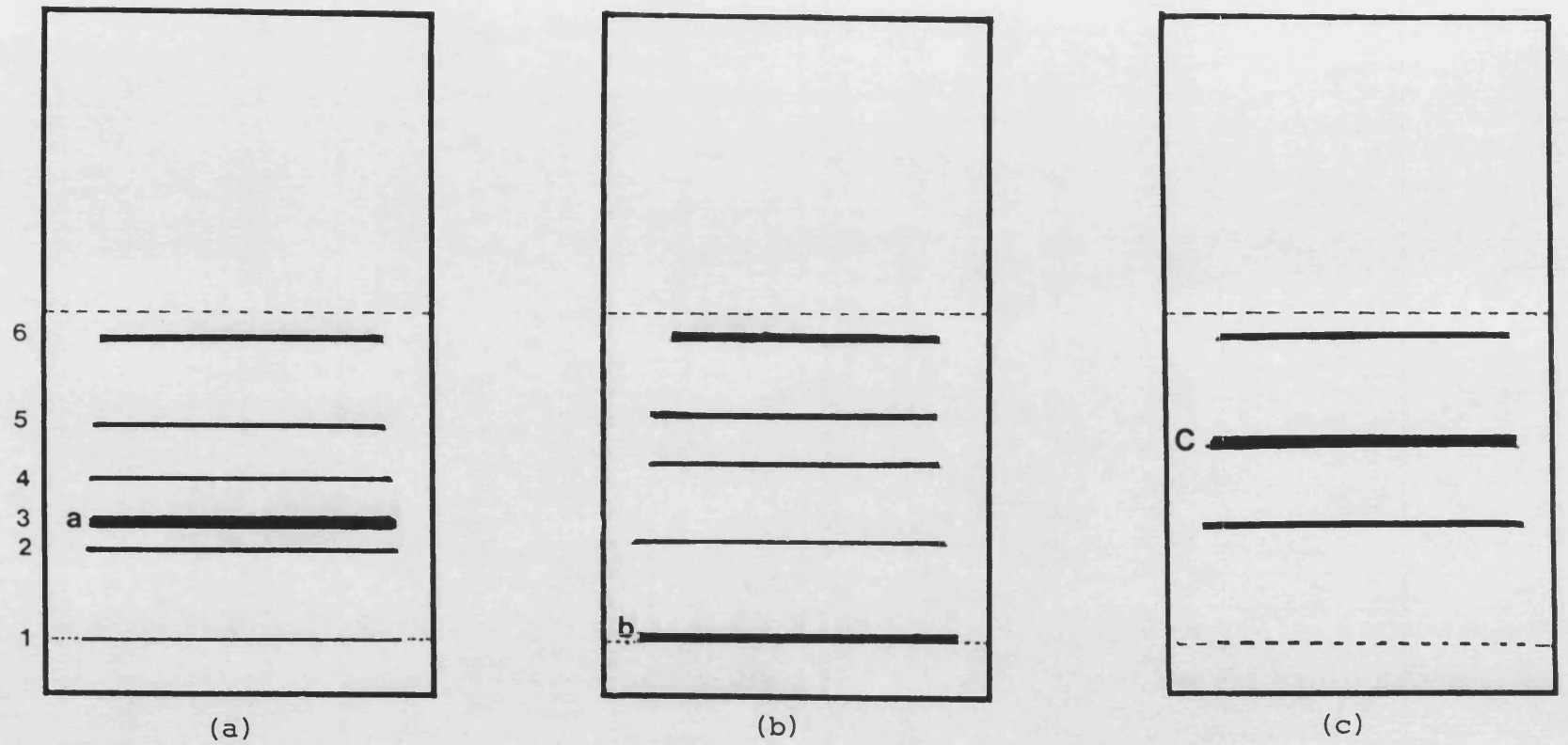


Fig. 3.7.5 Preparative T.L.C. Chromatograms for Irradiated Hydrocortisone (a), Hydrocortisone Phosphate (b) and Hydrocortisone Acetate (c) for 40 K.Gray in Propylene Glycol Detected under u.v. Light at 254 nm and 366 nm.

Table 3.7.4 DATA SHOWING R_f VALUES FOR HYDROCORTISONE, HYDROCORTISONE PHOSPHATE AND HYDROCORTISONE ACETATE AND THE POSSIBLE DEGRADATION PRODUCTS SEPARATED BY PREPARATIVE STRAIGHT PHASE TLC

Observed Bands	R_f Value		
	Hydrocortisone	Hydrocortisone Phosphate	Hydrocortisone Acetate
1	0 (366nm)	0	-
2	0.254 (366 nm only)	-	-
3	0.322	0.312	0.345 (366nm)
4	0.466	0.508	-
5	0.618	0.651	-
6	0.847 (366nm)	0.875 (366nm)	0.872 (366nm)
Observed R_f Values for Unirradiated Corticosteroids	0.322	0	0.572

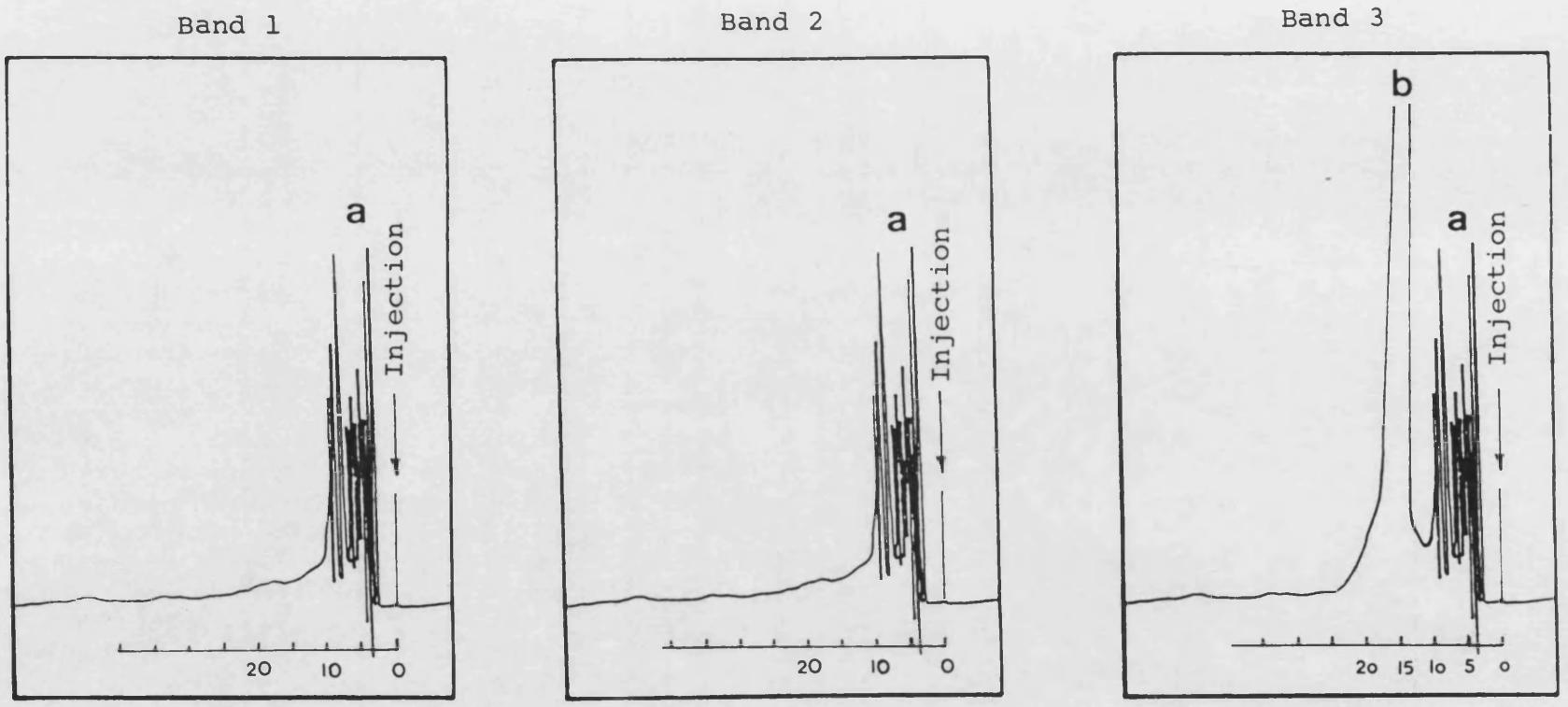


Fig. 3.7.6 HPLC Trace of the Extracted Bands of the Irradiated Solution of Hydrocortisone in Propylene Glycol

(a) Impurities of Silica Gel (b) Hydrocortisone

Chromatographic Conditions: Temperature: Ambient Flow Rate: 1ml/min.

Chart Speed: 0.2cm/min.

Absorbance Range: 0.05 AUFS at 248 nm.

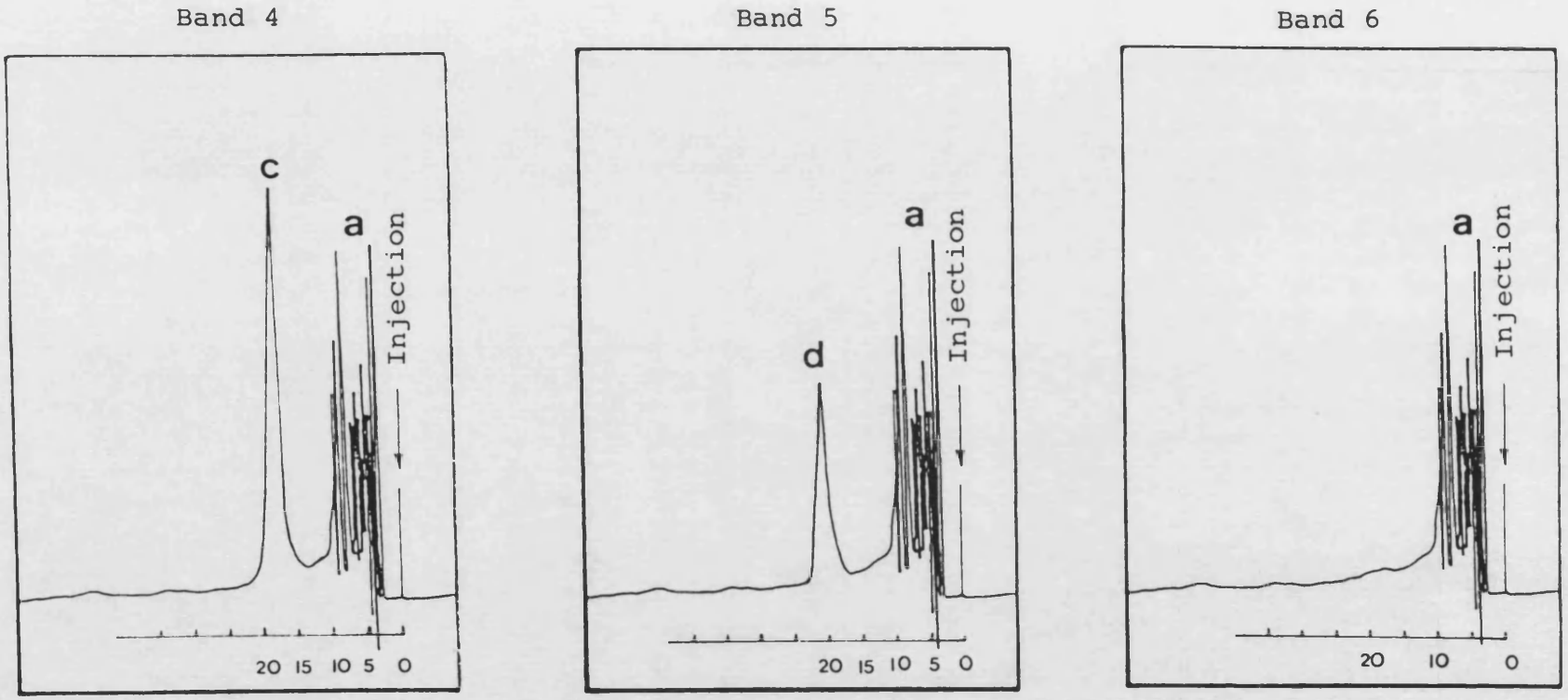


Fig. 3.7.6. (cont.) HPLC Trace of the Extracted Bands of the Irradiated Solution of Hydrocortisone in Propylene Glycol

(a) Impurities of Silica Gel (c), (d) Degradation Products

Chromatographic Conditions: Temperature: Ambient Flow Rate: 1 ml/min.

Chart Speed: 0.2cm/min.

Absorbance Range: 0.05 AUFS at 248 nm.

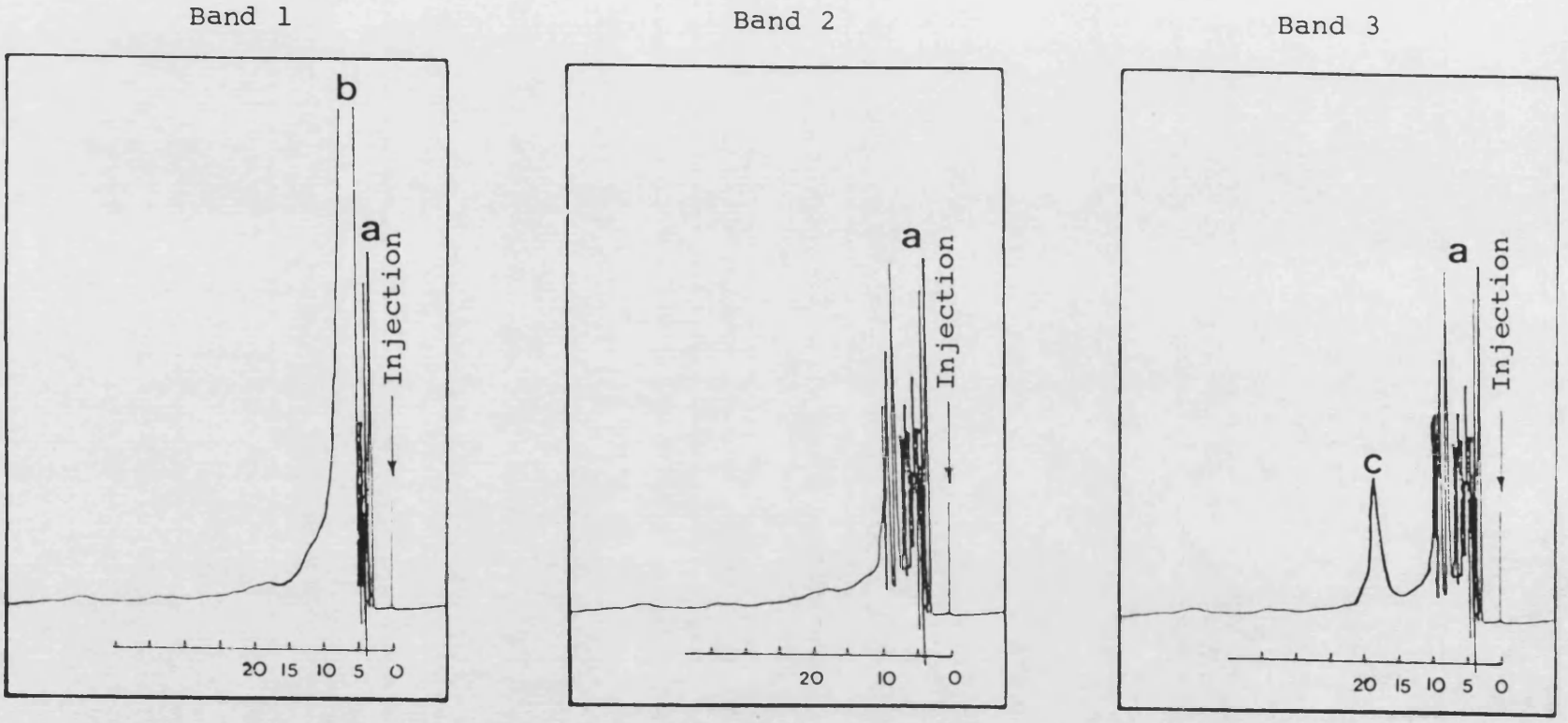


Fig. 3.7.7 HPLC Trace of the Extracted Bands of the Irradiated Solution of Hydrocortisone Phosphate in Propylene Glycol

(a) Impurities of Silica Gel (b) Hydrocortisone Phosphate (c) Degradation Product

Chromatographic Conditions: Temperature: Ambient Flow Rate: 1 ml/min.

Chart Speed: 0.2cm/min.

Absorbance Range: 0.05 AUFS at 248 nm.

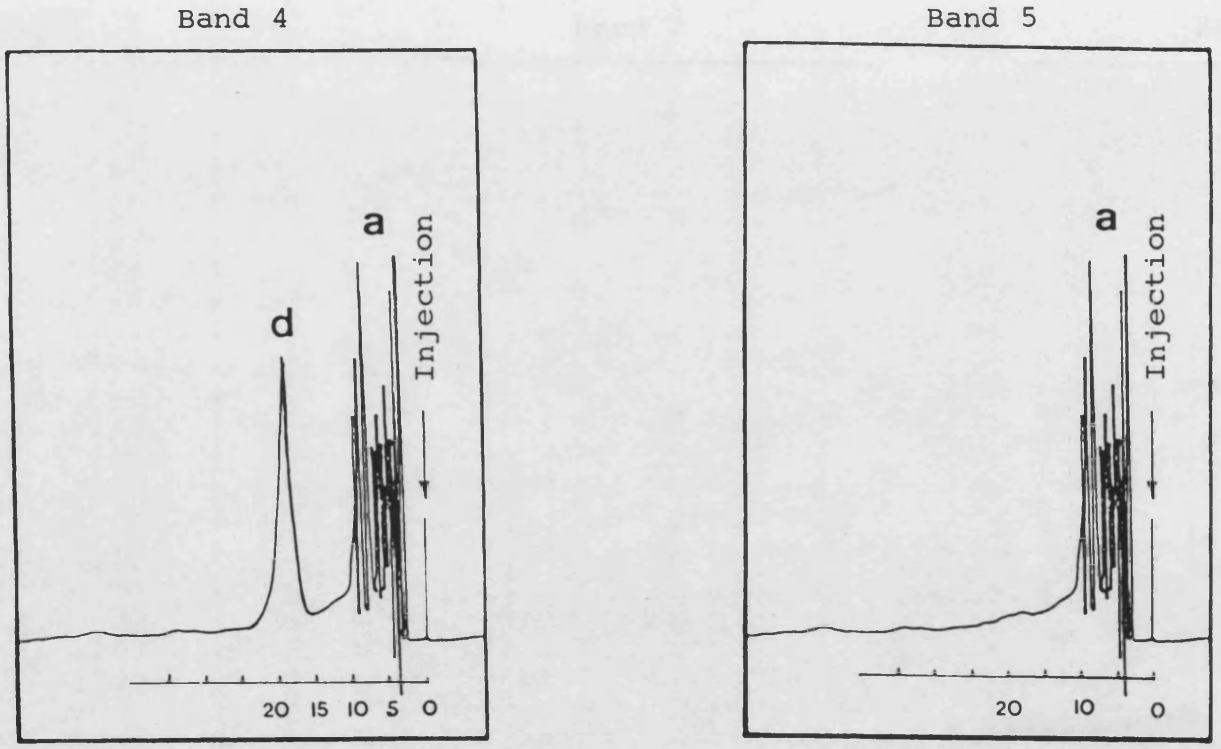


Fig. 3.7.7 (cont.) HPLC Trace of the Extracted Bands of the Irradiated Solution of Hydrocortisone Phosphate in Propylene Glycol.

(a) Impurities of Silica Gel (d) Degradation Product

Chromatographic Conditions: Temperature: Ambient Flow Rate: 1 ml/min.

Chart Speed: 0.2cm/min.

Absorbance Range: 0.05 AUFS at 248 nm.

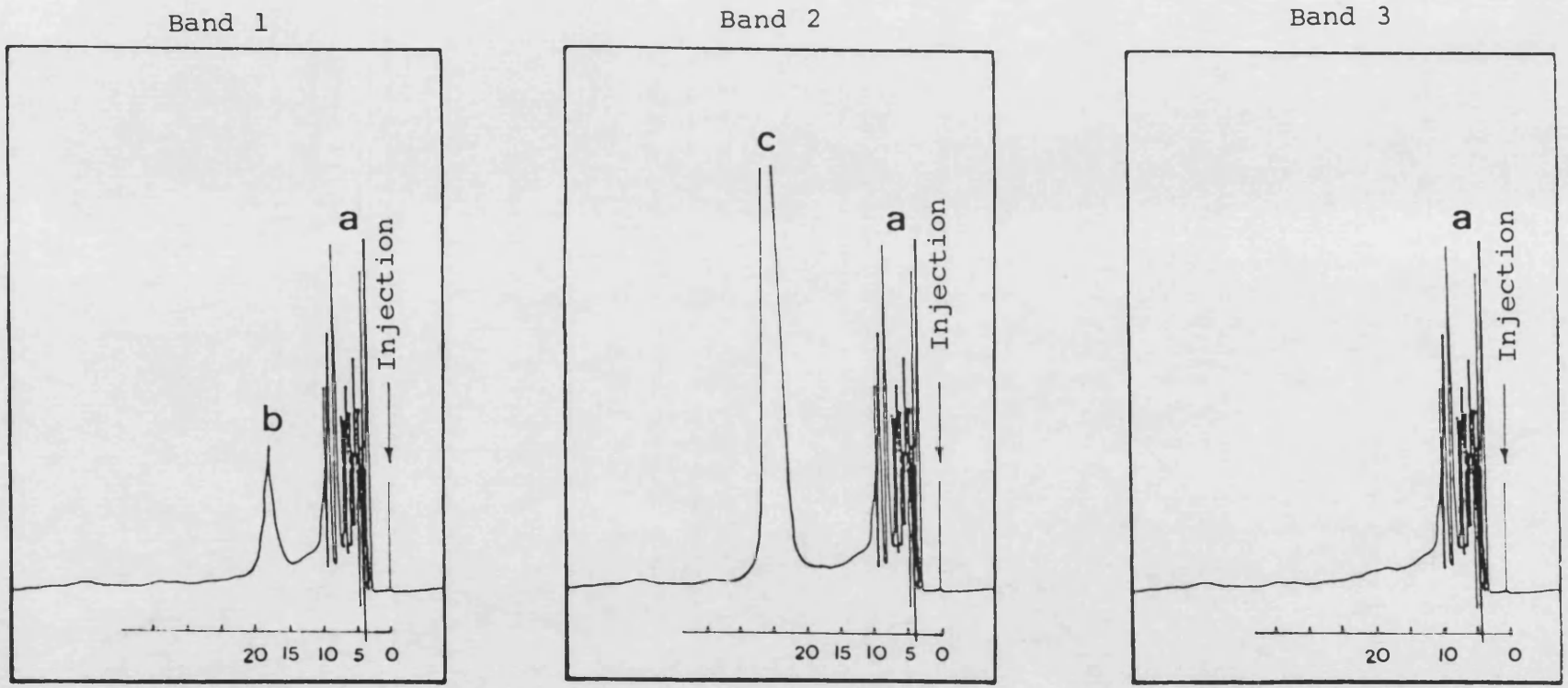


Fig. 3.7.8 HPLC Trace of the Extracted Bands of the Irradiated Solution of Hydrocortisone Acetate in Propylene Glycol

(a) Impurities of Silica Gel (b) Degradation Product (c) Hydrocortisone Acetate

Chromatographic Conditions: Temperature: Ambient Flow Rate: 1 ml/min.
Chart Speed: 0.2cm/min.
Asorbance Range: 0.05 AUFS at 248 nm.

4. DISCUSSION

DISCUSSION

The experimental work that is described in this Thesis can be divided into the following sections and will be treated as such for the purpose of discussion:

1. The assay procedures for the determination of hydrocortisone, hydrocortisone acetate and hydrocortisone phosphate in the presence of their radiolytic products.

2. Determination of the order of reaction for each of the three corticosteroids and subsequent determination of their respective G^- values.

3. The sensitivity of hydrocortisone and hydrocortisone phosphate to gamma-radiation in organic and aqueous solvents respectively.

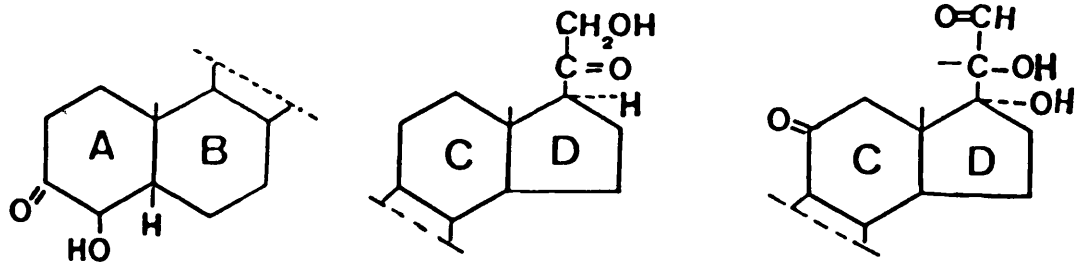
4. The effect of free radical scavengers on the sensitivity of the corticosteroids to gamma-radiation in the respective solvents.

5. The effect of surfactants on the sensitivity of corticosteroids in aqueous solutions to gamma-radiation.

6. The effect of gamma-radiation on formulated preparations of hydrocortisone.

7. Studies on the radiolytic degradation products of hydrocortisone, hydrocortisone acetate and hydrocortisone phosphate.

The assay method for determining a corticosteroid in the presence of its degradation products must be sensitive, selective and reproducible. The standard British Pharmacopoeia assay method[1,135] depends on measuring the absorbance of the coloured complex formed by reaction of an α-keto group with a neighbouring - OH group on the steroid molecule with the alkaline tetrazolium blue reagent. However, this method was considered to be insufficiently selective and specific for three reasons. Firstly, because most reducing steroids with a $-COCH_2OH$ side chain would react with the reagent to give the coloured complex. Secondly, esters of steroids would also give positive results as they are hydrolysed in the reaction mixture before the coloured complex is formed. Thirdly, the following possible degradation products of hydrocortisone would be capable of giving positive results with the reagent.



Therefore, the presence of any of these compounds would probably give a false indication of the residual concentration of the corticosteroid under investigation. A chromatographic method of separating the corticosteroid from its degradation products was therefore considered to be the most suitable approach for assaying the drug in the presence of its degradation products. HPLC techniques for corticosteroid separation and analysis are well documented in the literature[136-153] and were considered to form a suitable basis for the selection of a revelant assay method for the study. Because of the considerable difference in polarity between hydrocortisone, hydrocortisone acetate and hydrocortisone phosphate, a single standard assay for the three corticosteroids, under investigation, could not be achieved by means of one mobile phase. A reverse-phase HPLC system with a non-polar bonded stationary phase spherisorb S 10 ODS was used and found to be satisfactory when eluting with two different systems of mobile phase. For hydrocortisone and hydrocortisone acetate, reasonable separations were obtained as shown in figures 3.2.3 and 3.2.4 using mobile phases consisting of acetonitrile to water as 35 : 65 and 40 : 60 respectively, using hydrocortisone acetate as an internal standard for hydrocortisone and deoxycorticosterone for hydrocortisone acetate. In the case of hydrocortisone phosphate which is much more polar than hydrocortisone, another system consisting of methanol: 0.09M KH_2PO_4 (40 : 60) was used as a mobile phase and a suitable separation from the internal standard

prednisolone sodium succinate was obtained as shown in figure 3.2.7. Detection was by u.v. at 248 nm, the wavelength of maximum absorption for the three corticosteroids determined from figure 3.2.2. The calibration curves for hydrocortisone, hydrocortisone acetate and hydrocortisone phosphate, obtained from plotting the peak height ratio against the concentration of corticosteroid and shown in figures 3.2.5, 3.2.6 and 3.2.8 show that the ratios of slope to the standard deviation of slope were all much greater than 20 and the correlation coefficients are also highly significant at the 95% confidence level indicating good linearity. The intercepts are within two standard deviations of the zero point on the Y axis and the calibration curves can therefore be considered to pass through the origin. A Bartlett test, tables 3.2.2, 3.2.3 and 3.2.4, showed no significant difference between either the slope or the intercept values. Therefore, the assay method can be considered to be reproducible and suitable for determining quantitatively the residual concentrations of the three corticosteroids after exposure to gamma-radiation.

It has been reported that room temperature variations can affect the column performance, resulting in peak shape changes that adversely affect the assay precision[136,148]. Therefore, either instituting column temperature control or referring to a control of the unirradiated samples must be followed to ensure the reproducibility of the assay procedure. Throughout this work all the irradiated samples

were referred to unirradiated samples of the same solution.

It is evident from figures 3.7.1, 3.7.2 and 3.7.3 that the degradation products resulting after irradiation of the corticosteroids in the respective solvents, which are absorbed at 248 nm, are very small to interfere significantly with the peak of the parent drug. A confirmatory test which could be done to ensure that there are no peaks of degradation products under that of the parent drug is through injecting a sample of the irradiated solution of the corticosteroid onto the HPLC column coupled with a u.v./vis. spectrophotometer. As the corticosteroid peak elutes, a u.v. scan can be carried out every 3 seconds and the multiple scans are compared to a standard corticosteroid scan[149]. However, as the degradation products detected at 248 nm are in such small concentration that they would not contribute significantly to the peak height of the parent drug, a decrease in the peak height ratio of the corticosteroid under investigation would correspond to a decrease in its concentration.

In studying the effect of different types of surfactants on the sensitivity of corticosteroids to gamma-radiation, the possible interference of the surfactants to the assay procedure of the corticosteroids was investigated by determining calibration curves for the corticosteroids in the presence of different concentrations of surfactants. It is evident from the results presented in tables 3.5.1 and 3.5.2 that the surfactants do not interfere with the assay.

It is clear from figures 3.3.1, 3.3.2 and 3.3.3 that the concentration of hydrocortisone and hydrocortisone acetate in propylene glycol or hydrocortisone phosphate in water gradually decreases when subjected to increasing doses of gamma-radiation. The same figures also show that near-linearity only occurs in the initial portions of the curves, after which there is a tendency for the reaction rate to decrease.

The slopes of tangents drawn at zero dose of radiation are plotted on a log scale against log initial concentration of corticosteroid in figure 4.1 and the slopes obtained from the least square regression analysis were found to be 0.134, 0.096 and 0.065 for hydrocortisone, hydrocortisone acetate and hydrocortisone phosphate respectively, which suggest that the initial reaction rates of the three corticosteroids follow nearly zero order kinetics as already indicated by those calculated through the computerised least squares regression analysis presented in figure 3.3.4.

However, as shown in figure 4.2, the reaction rate of hydrocortisone seems to decrease as the dose of radiation increases, which would suggest that the reaction rate is not likely to be following true zero order kinetics. Therefore, if one compares the initial G^- values obtained from these curves to those at concentrations equivalent to the inital concentration reached after irradiation as indicated in figure 4.2, it is evident that the G^- values

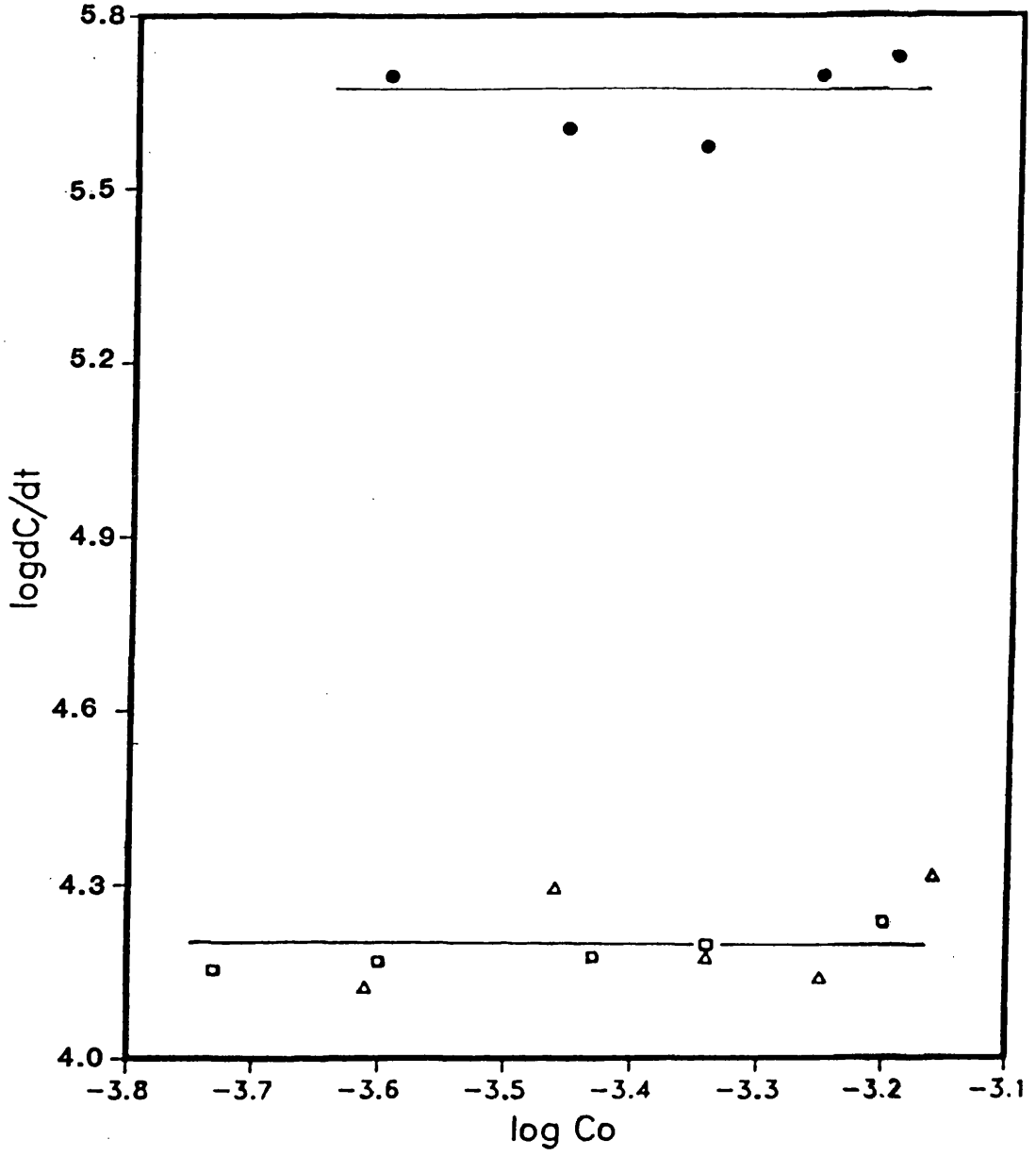


Figure 4.1 Plot of log $\frac{dc}{dt}$ Against log C_o in the Determination of the Order of Reaction for the Three Corticosteroids

● Hydrocortisone Phosphate

□ Hycrocortisone Acetate

△ Hydrocortisone

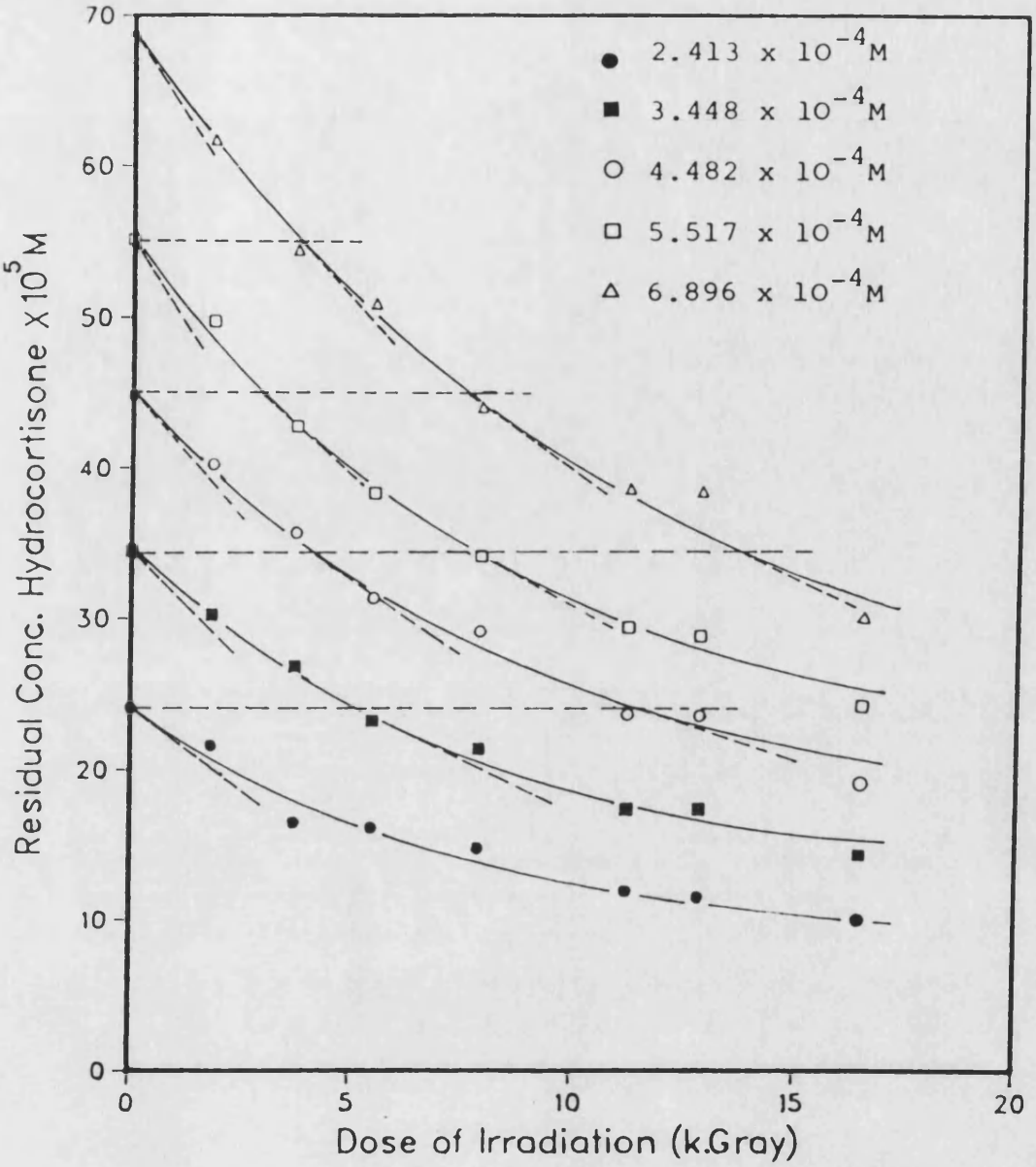


Figure 4.2 Plot of Residual Concentrations of Hydrocortisone in Propylene Glycol Against Dose of Irradiation for the Determination of the Reaction Rates at Concentrations Equivalent to the Initial Concentrations Reached After Irradiation

Table 4.1 DATA SHOWING THE G^- VALUES OF HYDROCORTISONE, HYDROCORTISONE ACETATE DEGRADATION IN PROPYLENE GLYCOL AND HYDROCORTISONE PHOSPHATE DEGRADATION IN WATER AT CONCENTRATIONS EQUIVALENT TO THE INITIAL CONCENTRATIONS REACHED AFTER IRRADIATION

CONCENTRATION OF CORTICOSTEROID x 10^4M	G^- VALUE				
	CURVE NUMBER				
	1	2	3	4	5
Hydrocortisone:					
6.896	0.392	-	-	-	-
5.517	0.263	0.299	-	-	-
4.482	0.207	0.249	0.316	-	-
3.448	0.152	0.186	0.228	0.380	-
2.413	-	0.065	0.119	0.174	0.293
Hydrocortisone Acetate:					
6.180	0.336	-	-	-	-
4.490	0.225	0.328	-	-	-
3.708	0.158	0.194	0.298	-	-
2.472	-	0.082	0.139	0.312	-
1.850	-	-	0.052	0.155	0.296
Hydrocortisone Phosphate:					
6.166	3.43	-	-	-	-
5.426	2.96	3.28	-	-	-
4.439	2.39	2.59	2.79	-	-
3.452	1.85	2.06	2.15	2.86	-
2.466	1.54	1.78	2.00	2.20	3.31

obtained after irradiation (i.e. in the presence of degradation products) are usually lower than the initial G^- values as shown in table 4.1. Similar results have been obtained for hydrocortisone acetate and hydrocortisone phosphate as shown in the same table. Also, if the G^- values at different doses of radiation, e.g. 5, 10 and 15 K.Grays, are calculated and compared to the initial G^- values, as shown in figure 4.3 and table 4.2, it can be seen that the G^- values decrease as the dose of radiation increases. Plotting the obtained data as log % residual concentration of corticosteroid against dose of radiation as shown in figure 4.4 and table 4.3, gives straight lines, which would support that the degradation of the drug over the range of radiation doses seems to be following first order Kinetics. It would appear therefore that the apparent inital zero order reaction has changed to a first order reaction after irradiation, which is surprising.

Generally, the Kinetics of radiation-chemical reactions are determined by competition of different solutes for the reaction with radicals, and the new radical formed is usually less reactive than the original radical[16]. So, if one kind of radical reacts with only one substance present in a solution, the quantity of substance reacting will be equal to the number of radicals formed, and hence proportional to the total radiation dose and independent of the concentration of the reacting solute. This is

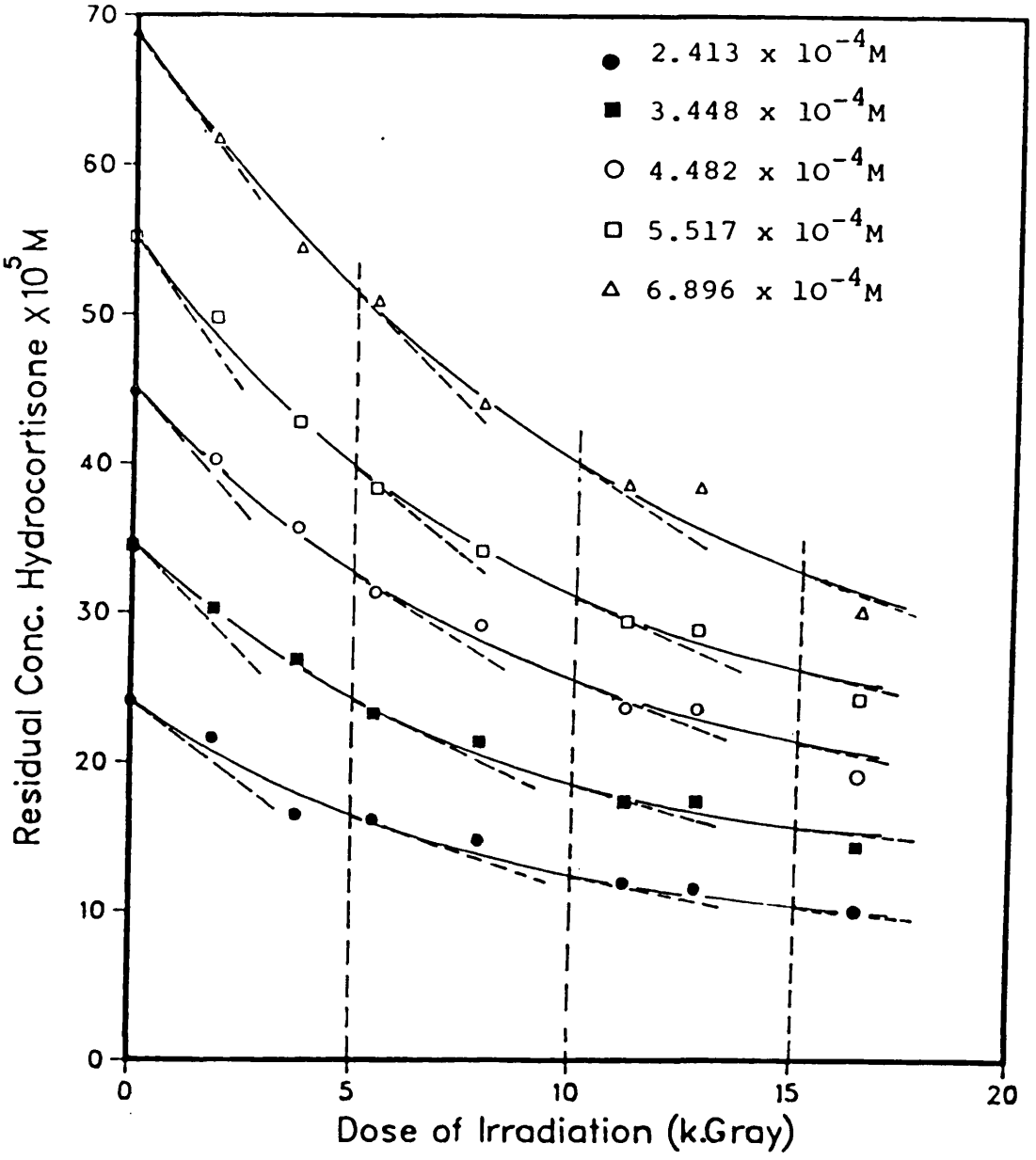


Figure 4.3 Plot of Residual Concentrations of Hydrocortisone in Propylene Glycol Against Dose of Irradiation for the Determination of the Reaction Rates at Different Doses of Radiation

Table 4.2 DATA SHOWING THE G^- VALUES OF HYDROCORTISONE (a), HYDROCORTISONE ACETATE (b) DEGRADATION IN PROPYLENE GLYCOL AND HYDROCORTISONE PHOSPHATE (c) DEGRADATION IN WATER AT DIFFERENT DOSES OF RADIATION

	DOSE OF RADIATION (K.Gy)	G^- VALUE				
		CURVE NUMBER				
		1	2	3	4	5
a	0	0.392	0.299	0.316	0.380	0.293
	5	0.251	0.160	0.151	0.177	0.104
	10	0.186	0.121	0.123	0.112	0.075
	15	0.127	0.065	0.074	0.065	0.053
b	0	0.336	0.328	0.298	0.312	0.296
	5	0.319	0.235	0.213	0.192	0.145
	10	0.180	0.189	0.139	0.065	0.035
	15	0.132	0.118	0.086	0.025	0
c	0	3.43	3.28	2.79	2.86	3.31
	1	2.65	2.62	2.16	1.85	1.85
	2	2.22	2.10	1.85	0.962	0.488
	3	1.70	1.52	1.15	0.695	0.192

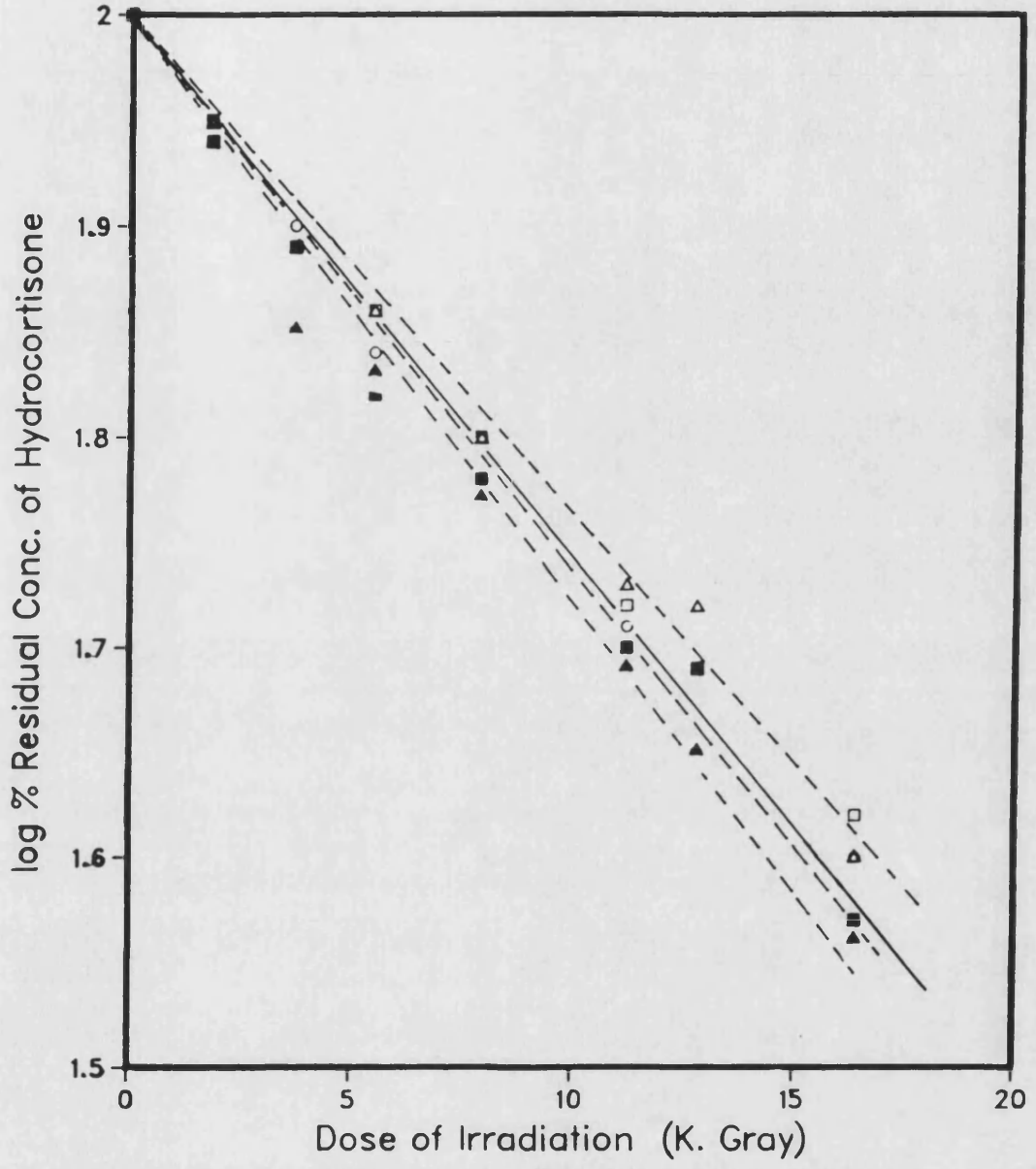


Figure 4.4 First-Order Plot for Different Initial Concentrations of Hydrocortisone Degradation in Propylene Glycol by Ionising Radiation

Table 4.3 THE LEAST SQUARES REGRESSION ANALYSIS DATA FOR PLOTTING % RESIDUAL CONCENTRATIONS OF CORTICOSTEROIDS AGAINST DOSE OF RADIATION

INITIAL CONCENTRATION OF CORTICOSTEROID x 10^{4}M	SLOPE	S.D. OF SLOPE	SLOPE / S.D.	CORRELATION COEFFICIENT
Hydrocortisone:				
6.896	-0.02307	0.00092	25.03	0.9951
5.517	-0.02290	0.00099	23.02	0.9944
4.482	-0.02410	0.00067	35.90	0.9974
3.448	-0.02492	0.00111	22.30	0.9939
2.413	-0.02612	0.00119	21.95	0.9939
Hydrocortisone Acetate:				
6.180	-0.01741	0.00106	16.36	0.9889
4.490	-0.01842	0.00134	13.77	0.9843
3.708	-0.02086	0.00178	11.67	0.9787
2.472	-0.01844	0.00300	6.15	0.9289
1.850	-0.02369	0.00437	5.42	0.9110
Hydrocortisone Phosphate:				
6.166	-0.16711	0.04971	3.36	0.7854
5.426	-0.19041	0.05844	3.26	0.7765
4.439	-0.20082	0.05818	3.45	0.7937
3.452	-0.19711	0.04781	3.18	0.8449
2.466	-0.19884	0.06485	3.07	0.7569

actually the case in the initial degradation of the three corticosteroids with the exception that the corticosteroid may be attacked by more than one species. Therefore, the reaction rate, initially, follows approximately zero order Kinetics.

In complex reacting systems, significant intermediates will fall into one or more of four groups of unstable species: 1) unstable molecules, 2) free radicals, 3) ions and 4) electronically, vibrationally and/or rotationally excited species[162,163], and the reaction rate can be expressed as:

$$\text{Rate of Reaction} = K.A^{\alpha}.B^{\beta}.C^{\gamma}$$

where α, β and γ are the order of the reaction with respect to species A, B and C. Initially, A is the only concentration that changes appreciably during the reaction and the rate can be given by the equation:

$$\text{Reaction Rate} = K\,A^{\alpha}$$

where α can be determined by varying the initial concentration.

A complex mechanism may occur in parallel or consecutive steps. The consecutive steps could be depicted schematically thus:

$$R \xrightarrow{K_o} I, \xrightarrow{K_1} I_2 \xrightarrow{K_2} \ldots\ldots \xrightarrow{K_{n-1}} I_n \xrightarrow{K_n} P \ldots\ldots \quad 4.1$$

where R, I and P are one or more reactant, intermediate and product molecules respectively. Some molecules represented by R, I or P may be spectators in a particular step. In a parallel mechanism, however, two or more sequences similar

to that in equation 4.1 will proceed along two or more different reaction coordinates simultaneously. It is usually more common to have a combination of both parallel and sequential steps.

During the irradiation of the corticosteroids in propylene glycol or water, the corticosteroid can be expected to be attacked by more than one species simultaneously giving more than one intermediate or product. It has been reported[2,3,56,58] that the degradation of corticosteroids by gamma-radiation may follow more than one pathway either in ring A, C, D or in the side chain on ring D. On the other hand, the large number and small quantities of degradation products separated by TLC shown in figures 3.7.3 and 3.7.4 suggest that a consecutive mechanism is also probably occurring as the dose of radiation is increased. A chain reaction mechanism is not likely to be taking place as the yield values of the corticosteroids' degradation are continuously decreasing during irradiation, whereas in a chain reaction the yield should continuously increase over the course of degradation ultimately resulting in a high G^{-} value.

Therefore, as both consecutive and parallel mechanisms are likely to be taking place during the irradiation of the corticosteroids, a complex reaction could be the reason for an indirect change in the order of reaction from an initial zero order to a first order at higher doses of radiation.

In terms of relevance for pharmaceuticals, the initial G^- value is of specific importance as it represents the highest G^- value during exposure of the drug to radiation. Therefore, the initial G^- value will be used as an indication to the rate of reaction of the corticosteroids to gamma-radiation under different conditions throughout the whole work.

Comparing the initial G^- values of hydrocortisone, hydrocortisone acetate in propylene glycol presented in tables 3.3.1 and 3.3.2 to thoseof hydrocortisone phosphate in water presented in table 3.3.3, it is apparent that the mean G^- values obtained for hydrocortisone and hydrocortisone acetate in propylene glycol were 0.215 and 0.187 respectively while the mean G^- value of hydrocortisone phosphate in water was found to be 1.77 i.e. the sensitivity of hydrocortisone phosphate in water to gamma-radiation appears to be about 9 times that of hydrocortisone or hydrocortisone acetate in propylene glycol. This indicates the greater reactivity of the corticosteroid to the radiolytic products of water than its reactivity to the radiolytic products of propylene glycol.

However, to compare the sensitivity of corticosteroids in different solvents, one drug ideally should be investigated in the proposed solvents. Therefore, being soluble in both aqueous and organic solvents, hydrocortisone phosphate was selected for this purpose. It is evident from figure 3.3.5 that the degradation of hydrocortisone phosphate in water due to irradiation is greater than its degradation in methanol or propylene glycol. However, the drug is more sensitive to

radiation in propylene glycol than in methanol. The apparent higher sensitivity of the drug in propylene glycol to radiation than in methanol could be due to the probable larger number of organic radicals as shown in table 1.1 produced through irradiation of propylene glycol, which could then attack the drug. This larger number of radicals could be a result of the larger hydrocarbon chain length of propylene glycol compared to that of methanol. Also, the two hydroxyl groups in propylene glycol may facilitate the loss of hydrogen atoms attached to the α-carbon atom[30,31,32] and therefore produce more radicals than methanol which has only one hydroxyl group.

It is clear from table 3.3.4 that the G^- value of hydrocortisone phosphate in water due to irradiation is about 17 times that in propylene glycol and about 66 times that in methanol. This result shows the higher sensitivity of the drug to radiation in aqueous solution than in organic solvents indicating the highly reactive nature of the radiolytic products of water.

As reported by many workers[20,21,23,28] the primary chemical result of irradiation of water after deposition of energy can be summarised as:

$$H_2O \longrightarrow \dot{H},\ \bar{e}_{aq.},\ \dot{O}H,\ H_3\overset{+}{O},\ H_2 \text{ and } H_2O_2$$

The yield values of hydroxyl radical, hydrated electron and hydrogen atom are 2.6, 2.8 and 0.6 respectively, whereas the yield of hydrogen peroxide and molecular hydrogen are 0.71 and 0.45. Attention was therefore focused on the effect of the first three radiolytic products as they were considered to be the most reactive species.

Many investigations have been reported on the reactions of the radiolytic products of water with different organic compounds and pharmaceuticals. For example, the reactions of the hydroxyl radical with simple organic molecules such as phenols and aniline have been studied by Neta et al and other workers[46-52] and the organic radicals produced were found to be as a result of the addition of the hydroxyl radical to the aromatic ring. Also, pulse radiolysis to toluene and aromatic compounds, containing a side chain such as benzyl chloride, bromide or acetate, has shown that the hydroxyl radical abstracts, preferentially, the hydrogen atom from the side chain[51].

The interaction of the hydrated electron with simple organic compounds such as phenylalanine has been studied by Hayon and Allen[52,53] who found that both deamination and addition to the aromatic ring occurs. The degradation of sulfonamides in aqueous solutions by gamma-radiation has been investigated by Phillips et al[4,8] who concluded that all the degradation effects were initiated by a reaction with the hydrated electron as well as the hydroxyl radical and the G^- value varied from 3.5 - 5.1. Similar results have been reported by Tsuji et al[5] through his study on the degradation pathways of penicillin G, neomycin, novobiocin and dihydrostreptomycin. The sensitivity of steroids to radiation has been studied by Allinson and Scholes[56,57] who irradiated aqueous solutions of cortisone and deoxycorticosterone and found that the radiolytic products of water attacked the steroid molecules resulting in reactions at different locations, such as the addition

of the hydrogen atom, the hydroxyl radical or the hydroperoxy radical in ring A which results in partial or complete reduction at the site of addition. Attack on the side chain of the steroid molecule may also take place, as shown by the same authors, and leads to the abstraction of one or two of the hydroxyl groups at that location. However, Coleby et al[59] have reported that, in organic solvents such as methanol, the steroid molecule was found to be more stable than in aqueous solution when subjected to γ-radiation, and he suggested the site of attack was different from that in the aqueous solution.

In order to compare the effect of the radiolytic products of water, namely the hydrogen atom, the hydroxyl radical and the hydrated electron on hydrocortisone phosphate, it was decided to study the effect of each one of them individually. The effect of the hydroxyl radical was studied by preparing the solution of hydrocortisone phosphate in water which had been bubbled with nitrous oxide to convert the hydrated electron and the hydrogen atom to the hydroxyl radical, while the sensitivity of the corticosteroid to the hydrated electron was investigated by using an alkaline medium to convert the hydrogen atom to the hydrated electron and using methanol as a radical scavenger to eliminate the hydroxyl radical. The reactivity of the hydrogen atom to hydrocortisone phosphate was determined by converting the hydrated electron to a hydrogen atom in an acid medium and the hydroxyl radical to the hydrogen atom by bubbling the solution with hydrogen gas. The sensitivity of hydrocortisone

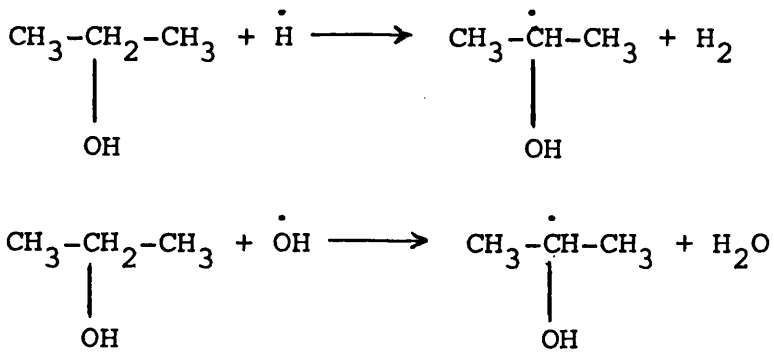
phosphate to the combined and individual radiolytic products of water is shown in figure 3.3.7, from which it can be observed that only about 3.95% of the drug was degraded by the hydrated electron at a dose of radiation of 2.29 K.Gray, while approximately 72% of the drug was degraded by the hydroxyl radical or hydrogen atom at the same dose of radiation. This indicates that the hydrated electron has only a very little destructive effect on the drug compared to that of the hydroxyl radical or hydrogen atom. However, it can also be seen that the destructive effect of the hydroxyl radical is slightly greater than that of the hydrogen atom as shown from the G^- values presented in table 3.3.5. However, it is possible that this observed small difference in figure 3.3.7 between the hydrogen atom's effect on hydrocortisone phosphate compared to that of the hydroxyl radical may be a result of an inadequate removal of the hydroxyl radicals from the system because of the low solubility of hydrogen gas in water (1 in 50 v/v at 0^oC). Therefore, what is being observed is partially a hydrogen atom effect and partially a hydroxyl radical effect. To ensure greater solubility of the hydrogen gas, the practical conditions should have been carried out at a reduced temperature and preferably under high pressure (>100 atmospheres). A more realistic method of answering the question would be to use a hydroxyl radical scavenger such

as tertiary butyl alcohol, and therefore give a clearer indication of the real effect of the hydrogen atom on the corticosteroid. Bacause of the high yields of the hydroxyl radical and the hydrated electron (G_{OH} = 2.6 and $G_{\bar{e}aq}$ = 2.8) resulting from water hydrolysis compared to those of the hydrogen atom, hydrogen peroxide and molecular hydrogen ($G_{H\cdot}$ =0.6, $G_{H_2O_2}$ = 0.71 and G_{H_2} = 0.45), it is likely that the degradation of hydrocortisone phosphate in aqueous solution is mostly due to the hydroxyl radical rather than the hydrated electron which is apparently much less reactive.

It has been reported that the addition of certain substances, in relatively small concentration, called scavengers, to a solution of organic material may markedly affect the radiolytic yield of that system through their reaction with the primary radiolytic products resulting from radiation[74]. For example, methanol acts as a scavenger for $\dot{O}H$ radical, $\dot{H}$ and hydrated electron resulting from radiolysis of aqueous solutions, while 2-propanol acts as a scavenger only for $\dot{O}H$ and $\dot{H}$ [27].

$$CH_3OH + \dot{H} \longrightarrow \dot{C}H_2OH + H_2$$

$$CH_3OH + \dot{O}H \longrightarrow \dot{C}H_2OH + H_2O$$



It can be observed from figures 3.4.5 and 3.4.6 that both methanol and 2-propanol have a protective effect on hydrocortisone phosphate when irradiated in aqueous solution. On increasing the percentage of both alcohols in aqueous solutions of hydrocrotisone phosphate, it is apparent from table 3.4.2 that 2-propanol is more effective as a stabiliser for the drug than methanol. This result is to be expected because 2-propanol is considered to be more reactive to $\dot{O}H$ and $\dot{H}$ than methanol as shown in table 1.2 [27]. This result would indicate the important role of $\dot{O}H$ and $\dot{H}$ radicals in the degradation of hydrocrotisone phosphate in the irradiated aqueous solution.

Using mixtures of different percentages of methanol and 2-propanol as scavengers for the radiolytic products in aqueous solution of the drug, it can be noted from figure 3.4.7 and table 3.4.3 that the mixture of both alcohols is more effective as a stabiliser for the drug than using each alcohol alone. The reason for this result

may be because the mixture combines the advantages of scavenging the hydrated electron by methanol along with the high reactivity of 2-propanol to both $\dot{O}H$ and $\dot{H}$, resulting in a more efficient scavenging effect of all three major radiolytic products of water and consequently providing more protection to the drug.

To investigate further the possible important role of the hydroxyl radical in the degradation of hydrocortisone phosphate in aqueous solution, oxygen was used as a radical scavenger which does not react with the hydroxyl radical and reacts only with hydrogen atom and hydrated electron as shown in table 1.2 [27].

$$O_2 + \bar{e}_{aq} \longrightarrow \bar{O}_2 \text{ ..... } 1.9 \times 10^{10} \text{ l mol}^{-1}\text{Sec}^{-1}$$

$$O_2 + \dot{H} \longrightarrow \dot{H}O_2 \text{ ..... } 2.2 \times 10^{10} \text{ l mol}^{-1}\text{Sec}^{-1}$$

From the obtained results shown in figure 3.4.10 and table 3.4.5 no effect was observed for oxygen on the sensitivity of hydrocortisone phosphate to radiation in aqueous solution. However, due to the low solutility of oxygen in water (1 in 32 v/v at 20°C), the hydrogen atom and the solvated electron are only partially scavenged by bubbling oxygen. Nevertheless, the removal of the naturally dissolved oxygen in water by bubbling helium does not affect the sensitivity of the corticosteroid as shown in figure 3.4.10 which would suggest that the hydroxyl radical is the main destructive species.

On the other hand, the presence of osygen in the aqueous solutions may result in an indirect removal of some of the hydroxyl radicals [26,27] according to the following equations:

$$O_2 + \bar{e}_{aq} \longrightarrow \dot{O}_2^-$$

$$\dot{O}_2^- + \dot{O}H \longrightarrow \bar{O}H + O_2$$

$$O_2 + \dot{H} \longrightarrow H\dot{O}_2$$

$$H\dot{O}_2 + \dot{O}H \longrightarrow H_2O_3$$

But it seems that the reaction of the hydroxyl radical with the steroid molecules is more energetically favoured than its reaction with the superoxide ion or hydroperoxy radical. All these results would suggest that primarily it is the hydroxyl radical which is possibly responsible for the degradation of the corticosteroid in aqueous solution.

From the results obtained in table 3.3.4 for comparing the effect of different solvents on the sensitivity of hydrocortisone phosphate to gamma-radiation, it is clear that there is a significant difference due to the sensitivity of the drug to γ-radiation in different organic solvents, where the G^- value of the drug in propylene glycol was about 3 times as great as that in methanol. This means that either the attacking species produced by radiolysis of propylene glycol are more reactive than those produced by methanol, or the propylene glycol itself is more sensitive to radiation than methanol, therefore more radiolytic species are produced which attack the corticosteroid, or a combination of both of these explanations is the reason for the apparent higher sensitivity of the drug in propylene glycol than in methanol.

Extensive studies have been carried out on the radiolysis of aliphatic alcohols[30-34] and it has been reported that all the studied alcohols gave alkoxy radicals and hydroxyalkyl radicals derived by the loss of a hydrogen atom from the carbon adjacent to the OH group (α-carbon). The alkoxy radical may be the precursor for the formation of the hydroxyalkyl radical:

$$CH_3\overset{+}{O}H + CH_3OH \longrightarrow CH_3\dot{O} + CH_3\overset{+}{O}H_2$$

$$CH_3\dot{O} + CH_3OH \longrightarrow \dot{C}H_2OH + CH_3OH$$

Generally, the alkyl chain length as well as the degree of branching largely affects the reactivity of alcohols[6]. The yield of hydrocarbons, aldehydes and glycols from primary alcohols decreases as the chain length of the alkyl chain increases, while the hydrogen yield remains constant, for example in radiolysis of methanol[30,31,32,35,36].

$$CH_3OH \sim\sim\longrightarrow \dot{C}H_2OH + \dot{H}$$

$$\dot{H} + CH_3OH \longrightarrow \dot{C}H_2OH + H_2$$

$$2\dot{C}H_2OH \longrightarrow \underset{\text{Glycol}}{(CH_2OH)_2}$$

$$\dot{C}H_2OH \longrightarrow \underset{\text{Aldehyde}}{CH_2O} + \dot{H}$$

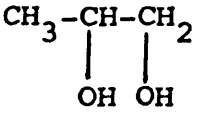
This would indicate that bonds are broken elsewhere in the molecule than the α-C, and it was found that the C-C bonds can also break quite readily as indicated by the

number of possible hydrocarbons produced.

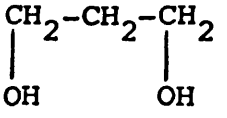
Radiolysis of glycols has also been studied[38] and it was found that a greater variety of radicals could be expected as shown in table 1.1 due to the presence of two hydroxyl groups in the molecule. Knowledge of the effect of the number of the hydroxyl groups in alcohol molecules on their sensitivity to radiation can be gained through the study of the influence of this factor on a series of aliphatic alcohols, with the same length of hydrocarbon chain and with different numbers and positions of hydroxyl groups. For example, n-propanol has one hydroxyl group, propylene glycol has two hydroxyl groups in positions 1 and 2 of the hydrocarbon chain, 1,3 propanediol has two hydroxyl groups in positions 1 and 3 and glycerol has three hydroxyl groups.

n-propanol $CH_3-CH_2-CH_2-OH$

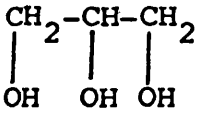
Propylene Glycol



1,3 propanediol



Glycerol



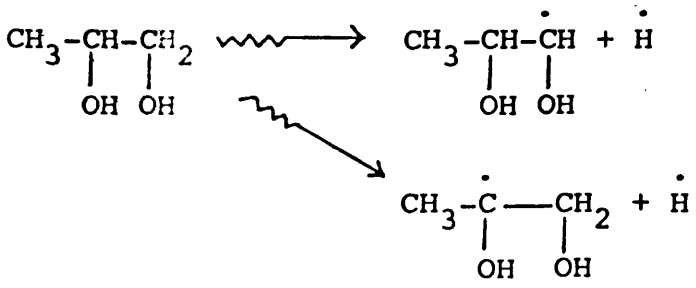
From the calculated G^- values shown in table 3.3.6, it is evident that the order of sensitivity of hydrocortisone in these alcohols to gamma-radiation is n-propanol < propylene glycol < glycerol < 1,3 propanediol, which means

that the sensitivity of these alcohols to radiation increases as the number of hydroxyl groups increases. Also, the position of the hydroxyl group in the alcohol molecule apparently affects the sensitivity of the alcohol to radiation, where the presence of hydroxyl groups in 1 and 3 positions results in more degradation of hydrocortisone in the case of 1,3 propanediol than in propylene glycol where the hydroxyl groups are in positions 1 and 2. The reactivity of the alcohol in this study is manifested through the effect of the produced radicals on the hydrocortisone dissolved in such alcohol.

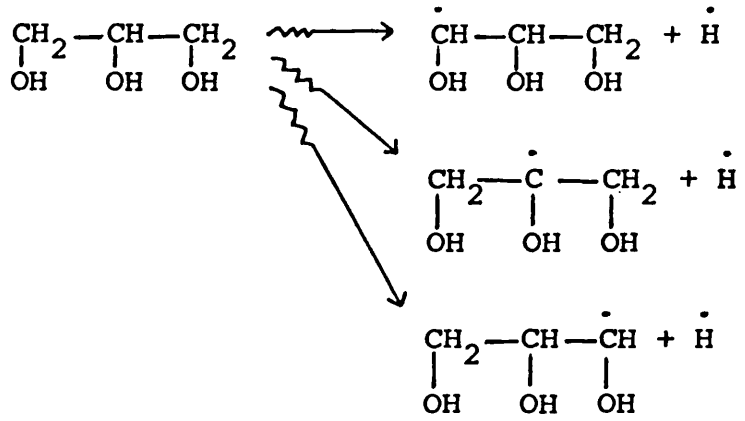
The effect of the number of hydroxyl groups on the reactivity of alcohols can be explained by the fact that an OH group increases the electron density on the adjacent carbon atom due to the electrophilic nature of the oxygen atom, and this high electron density would facilitate the abstraction of the hydrogen atom from that carbon giving an organic radical. For example, in the case of n-propanol, where there is only one hydroxyl group, the most likely formed organic radical is $CH_3-CH_2-\dot{C}HOH$

$$CH_3-CH_2-CH_2OH \rightsquigarrow CH_3-CH_2-\dot{C}HOH + \dot{H}$$

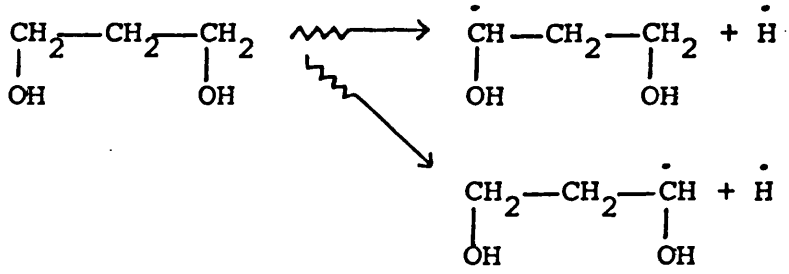
while in the case of propylene glycol, two probable organic radicals are likely to be formed



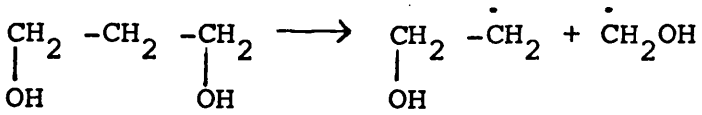
The number of probable radicals increases in the case of glycerol where three hydroxyl groups are attached to the hydrocarbon chain



In the case of 1,3 propanediol, the same possibilities of hydrogen atom abstraction as in propylene glycol may take place



However, the high reactivity of 1,3 propanediol can be explained in two ways. Firstly, the organic radicals produced by radiolysis of 1,3 propanediol may be more reactive than those produced by propylene glycol. Secondly, the presence of the hydroxyl groups at both ends of the 1,3 propanediol molecule may result in the production of high electron density at both ends which in turn may facilitate the fragmentation of the molecule and the production of other organic radicals as follows:



The possibility of this fragmentation is rather difficult in the case of propylene glycol or glycerol because the hydroxyl groups are located in the adjacent carbon atoms. Accordingly, this could be a possible explanation for the higher sensitivity of hydrocortisone to radiation in 1,3 propanediol than in propylene glycol and glycerol.

In addition to these possibilities of hydrogen atom abstraction from the alcohol molecules, the other possibilities of hydrogen atom and hydroxyl radical abstraction from other locations in the molecule may occur according to the same scheme shown for propylene glycol in table 1.1.

Using methanol as a radical scavenger, table 3.4.1, shows that the addition of 20% v/v of methanol to hydrocortisone solutions in propylene glycol, glycerol and 1,3 propanediol results in stabilisation of the corticosteroid to γ-radiation by 65%, 42% and 69% respectively which indicates that methanol interacts with the radiolytic products of these solvents, namely hydrogen atom, hydroxyl radical and organic radicals.

Although glycerol, as has already been shown in the radiolysis scheme of these three solvents, has the greatest possibility of $\dot{H}$ and $\dot{O}H$ loss by irradiation, it is nevertheless the least stabilised solvent by methanol. This could mean that the reactivity of the organic radicals produced by irradiation is the main factor which controls

the degree of stabilisation of the solvent by methanol. In other words, the organic radicals produced from propylene glycol and 1,3 propanediol by radiolysis are very similar in structure and hence have nearly the same reactivity to methanol. The small difference between propylene glycol and 1,3 propanediol could be due to the possible molecular fragmentation of the latter by irradiation giving more reactive organic radicals which may be more efficiently scavenged by methanol. In the case of glycerol the organic radicals resulting from irradiation may be less reactive to methanol than those produced by propylene glycol and 1,3 propanediol. Therefore their removal from the solution by methanol is not as efficient as the removal of propylene glycol and 1,3 propanediol radicals.

Figures 3.4.8, 3.4.9 and table 3.4.4 show that the removal of oxygen from the propylene glycol solutions, by bubbling with either helium or nitrogen, results in an increase in the degradation of hydrocortisone and hydrocortisone acetate whereas the saturation of the solution with oxygen results in greater stabilisation of the corticosteroids to gamma-radiation. This would suggest that oxygen must be reacting with some or all of the radiolytic products of propylene glycol namely $\dot{H}$, $\dot{O}H$ and the organic radicals in order to afford the observed protection of the corticosteroids to ionising radiation. It has already been noted from table 1.2 that oxygen does not react with $\dot{O}H$. This would mean that the probable

destructive reactants to the corticosteroid are the $\dot{H}$ and the organic radicals which are being removed by the oxygen to afford protection. However, it has been shown from studying the effect of oxygen on the sensitivity of hydrocortisone phosphate in aqueous solution to radiation that $\dot{H}$ preferentially reacts with the corticosteroid rather than with oxygen. One can therefore conclude that the radiolytic species of propylene glycol that are being scavenged by oxygen and therefore affording protection to the corticosteroids are most likely to be the organic radicals. Ahmed *et al*[39] who investigated the γ- radiolysis of ethylene glycol have reported the possible formation of organic peroxide as a result of the reaction of oxygen with the organic radicals.

$$O_2 + \dot{C}H(OH)CH_2OH \longrightarrow \dot{O}\text{-}O\text{-}CH(OH)CH_2OH$$

$$\dot{O}\text{-}O\text{-}CH(OH)CH_2OH \longrightarrow HO\text{-}CH_2\text{-}CHO + \dot{H}O_2$$

$$\dot{O}\text{-}O\text{-}CH(OH)CH_2OH + \dot{H}O_2 \longrightarrow HO\text{-}CH_2CHO + H_2O_2 + O_2$$

Similarly, oxygen may react with the organic radicals produced by radiolysis of propylene glycol resulting in the formation of organic peroxide as follows:

$$CH_3CH(OH)CH_2OH \rightsquigarrow CH_3CH(OH)\dot{C}HOH + \dot{H}$$

$$CH_3CH(OH)\text{-}\dot{C}HOH + O_2 \longrightarrow CH_3\text{-}CH(OH)\underset{\dot{O}\text{-}O.}{CHOH}$$

$$CH_3CH(OH)\text{-}\underset{O\text{-}\dot{O}}{CHOH} \longrightarrow CH_3CH(OH)CHO + \dot{H}O_2$$

$$CH_3CH(OH)\text{-}\underset{\dot{O}\text{-}O.}{CHOH} + \dot{H}O_2 \longrightarrow CH_3CH(OH)CHO + H_2O_2 + O_2$$

This scavenging of organic radicals could explain the protective effect of oxygen when hydrocortisone in propylene glycol is exposed to γ-radiation, and hence shows the important role of organic radicals in the degradation of the corticosteroids. Accordingly, it was decided to investigate the effect of an organic radical scavenger on the senstivity of hydrocortisone in organic solvents to γ-radiation.

Schuler[76] has reported the high efficiency of iodine as an organic radical scavenger because of the low activation energy for the reaction of organic radicals with iodine:

$$\dot{R} + I_2 \longrightarrow RI + \dot{I}$$

together with the relative chemical inertness of iodine itself and of the resultant radical (iodine atom) formed in the reaction. An example for the use of iodine as a scavenger for the suppression of free radical processes had been reported by the same author in his studies on 2,2,4 trimethylpentane when a G^+ value of 0.3 was observed for methane production in the presence of 0.009M I_2, while a G^+ value of 1.00 had been recorded in the absence of iodine.

From figures 3.4.11, 3.4.14 and from the calculated reaction rate presented in table 3.4.7, it is evident that iodine has a significant protective effect on hydrocortisone against radiation in both propylene glycol and glycerol, where the addition of 1×10^{-3}M of iodine resulted in a reduction of the reaction rate of hydrocortisone by 81% and 71% in propylene glycol and glycerol respectively.

Using methanol as a cosolvent for iodine dissolution in both propylene glycol, fig. 3.4.13 and glycerol, fig. 3.4.14, resulted in a reduction of the efficiency of iodine as a scavenger for the organic radicals which has been manifested as an increase in the reaction rate of the corticosteroid. This effect of methanol could be expected because some of the iodine is consumed by the organic radicals produced by methanol through radiolysis.

However, if this scavenging effect of iodine is the only mechanism in stabilising the corticosteroid, it would be expected that in aqueous solution, where no organic radicals are present, iodine should not have any effect on the sensitivity of the corticosteroid to radiation. But surprising results were obtained on adding iodine to aqueous solutions of hydrocortisone phosphate using either 3.01×10^{-3}M KI or 2% v/v methanol to aid its dissolution, as shown in figure 3.4.17 and table 3.4.9 where the reaction rate of hydrocortisone was reduced by 7% by the addition of 6×10^{-4}M I_2 to the aqueous solution of hydrocortisone phosphate containing 3.01×10^{-3}M KI, while the addition of the same concentration of iodine reduced the reaction rate of the corticosteroid in aqueous solution containing 2% v/v methanol by 12%. This means that iodine still has a significant stabilising effect beside the effect of KI and methanol.

It is well known that the presence of halide ions in irradiated aqueous solutions results in the removal of the $\dot{O}H$ radical as follows[131]:

$$\dot{OH} + I^- \longrightarrow \dot{I} + \bar{O}H$$

which could explain the apparent protective effect of KI on the sensitivity of hydrocortisone phosphate in aqueous solution to radiation as shown in table 3.4.8 and figure 3.4.16. Also, the role of methanol as an effective scavenger for the radiolytic products of water has been shown in table 1.2.

The suggested effect of iodine in aqueous solution could be due to the capture of the solvated electron ($\bar{e}aq.$) from the irradiated solution and the formation of iodide ion I^- which in turn reacts with the hydroxyl radical as follows:

$$I_2 + \bar{e}_{aq} \longrightarrow I_2^-$$

$$I^- + \dot{OH} \longrightarrow \dot{I} + \bar{O}H$$

This hypothesis of the transformation of iodine to iodide ions is supported by the results obtained in table 3.4.10 where the reaction rate of hydrocortisone phosphate in aqueous solution in the presence of certain concentrations of KI is compared to that obtained in the presence of a mixture of I_2 and KI prepared in such proportions to produce the same total concentration of I^- ions after irradiation. From such a comparison, it is evident that both systems had nearly the same reaction rate for hydrocortisone phosphate, and the little difference could be attributed to the energy consumed to convert the iodine molecule to iodide ions.

From this series of experiments, it is apparent that iodine may act through two possible mechanisms in protecting the corticosteroid against radiation. The first mechanism is through the scavenging of the organic radicals produced by radiolysis of the organic solvent, while the second possible mechanism is through the capture of the hydrated electron produced by the radiolysis of water and converting it into an iodide ion which in turn removes the hydroxyl radical resulting in the protection of the corticosteroid molecules.

All these results show the important role of $\dot{O}H$ in the degradation of corticosteroids in aqueous solution. The sensitivity of hydrocortisone to gamma-radiation in aqueous solutions containing CTAB, NaLS and cetomacrogol 1000 are shown in figure 3.5.1. It is evident from this figure that the two ionic surfactants have a considerable protective effect on hydrocortisone degradation by the radiolytic products of water as their concentration is increased, whereas the non-ionic cetomacrogol 1000 has only a marginal protective effect as its concentration is increased. The stability of hydrocortisone therefore increases but to a different extent depending on the type of surfactant and the order of this stabilising effect is NaLS $>$ CTAB $>$ Cetomacrogol 1000. From these results one might conclude that the reactivities of the surfactants to the radiolytic products of water would be in the same order and that what one is observing is a protective mechanism by preferential competition of the radiolytic products of water for the respective surfactant molecules compared to their

reactivities for hydrocortisone. However, it is obvious from table 1.3 that CTAB has a higher reaction rate with all the radiolytic products than NaLS especially below the CMC. Therefore the observed results in figure 3.5.1 would indicate that simple competitive reactivities of a surfactant for the radiolytic products of water is not the only factor which controls the degree of protection conferred on the corticosteroid.

Bakalik and Thomas[161] have reported that NaLS, CTAB and Brij (polyoxyethylene dodecyl ether) decompose when subjected to gamma-radiation through abstraction of a hydrogen atom from the hydrocarbon chain part of the surfactant molecule to produce a surfactant radical:

$$C_{12}H_{25}SO_4^- + \dot{O}H \longrightarrow C_{12}\dot{H}_{24}SO_4^- + H_2O$$

This would mean that, in the system, such surfactant radicals would react with hydrocortisone, therefore destroying it and possibly counteracting the protective effect of the surfactant and resulting in a net reduction in the protection. As the reaction rate between a surfactant and the radiolytic products of water increases, the production of surfactant radicals will increase resulting in further attacks on hydrocortisone molecules and consequently a more reduced protective effect on the drug will result. This may possibly explain the observed higher protective effect of NaLS than that of CTAB despite its lower reaction rate with the radiolytic products of water.

Al-Saden *et al*[123] have also found that gamma-irradiation of non-ionic surfactant solutions can lead to polyoxyethylene chain scission. The produced radiolytic radicals may also have some destructive effect on hydrocortisone molecules besides the original protective effect of the surfactant molecules which results in a low overall protection of the drug against radiation as shown in figure 3.5.1.

From figure 3.5.1 it can also be observed that as the concentration of NaLS or CTAB is increased, the G^- value of hydrocortisone degradation by the radiolytic products of water decreases until a certain concentration of the surfactant is reached. Beyond these respective concentrations of NaLS and CTAB, the G^- value of hydrocortisone degradation appears to remain constant. This change in the effect of surfactants on the reactivity of the radiolytic products of water with hydrocortisone occurs at a concentration of 5.2×10^{-3}M and 9.3×10^{-4}M for NaLS and CTAB respectively. It would appear therefore that both the anionic and cationic surfactants protect hydrocortisone against radiation until these respective concentrations are reached and then above these concentrations the ionic surfactants do not seem to have any additional protective effect. It is possible that this abrupt change in the effect of the anionic and cationic surfactants on the sensitivity of hydrocortisone to radiation is related to the micellisation of the surfactants and that the concentrations at which this change occurs should coincide with the CMC's of the two surfactants.

The CMC's of NaLS and CTAB in aqueous solutions at $25^{o}C$ are quoted in the literature as $8 \times 10^{-3}M$ and $9.8 \times 10^{-4}M$ respectively[95]. Although the observed concentrations of the anionic and cationic surfactants at which the abrupt change in their effect on the sensitivity of hydrocortisone are lower than these quoted CMC's in simple aqueous solutions, they are in the same order of magnitude. A number of possible reasons could account for these apparently lower CMC's of the surfactants in the irradiated hydrocortisone solutions. First of all, the presence of a solubilisate may result in a reduction in the CMC of the surfactant. For example, Shinoda[98] has reported that solubilised hydrocarbon increases the micelle size and also causes changes in the curvature of the micelle surface and the dimensions of the micelle, resulting in a decrease in the CMC of about 5-30%. Therefore, the presence of hydrocortisone which can possibly associate with a surfactant micelle may result in some reduction in the normal CMC's of both anionic and cationic surfactants. Secondly, the presence of hydrocortisone degradation products in the irradiated solution could also similarly be responsible for a lowering of the CMC from the original value in the aqueous solutions. Bakalik and Thomas[161] have shown that the surfactant radicals, formed by an abstraction of a hydrogen atom from the hydrocarbon chain of NaLS or CTAB, can associate with micelles. It is possible for these radicals to react therefore with each other to form a compound which would have a larger hydrocarbon unit than

the parent surfactant compound. The association of these radicals with a micelle and the possible presence of a compound with a larger hydrophobic component could be a third reason for the reduction in the CMC's of the surfactants.

To find out if these explanations were feasible, determinations of the CMC's of NaLS and CTAB solutions containing hydrocortisone before and after irradiation were carried out by means of surface tension measurements by the Wilhelmy plate method[160]. From tables 3.5.7a and 3.5.8a it can be seen that the CMC values obtained for CTAB and NaLS are close to the quoted literature CMC values and no significant difference in the CMC of the surfactants is observed before and after irradiation. However, there is a considerable reduction in the CMC's due to the presence of hydrocortisone where the CMC of the CTAB solutions have shifted from 9.6×10^{-4}M to 6.9×10^{-4}M with hydrocortisone present and for NaLS the shift is from 8×10^{-3}M to 6.6×10^{-3}M respectively. These results suggest that possible association of hydrocortisone with the surfactant micelle could be the main reason for the depression of the CMC's rather than the radiolytic degradation products of hydrocortisone or surfactant monomers.

Assuming that the apparent change in the effect of the NaLS and CTAB on the reaction rate of hydrocortisone with the radiolytic products of water does coincide with their CMC's then figure 3.5.1 shows that the radiolytic products of water react preferentially with the ionic

surfactant monomers below the CMC. At and above the CMC it would seem that the reaction of the radiolytic products of water with the surfactants has reached its maximum and a steady state prevails. This means that above the CMC the same number of molecules of hydrocortisone are destroyed by the radiolytic products of water and that any increase in the concentration of the ionic surfactants does not afford any additional protection for hydrocortisone.

In the case of the non-ionic surfactant cetomacrogol 1000 no abrupt change in the effect of the surfactant on the sensitivity of hydrocortisone to γ-radiation can be detected as shown in figure 3.5.1, which means that the surfactant, both below and above its CMC, continues to protect the corticosteroid against radiation to approximately the same extent.

On determining the CMC's of cetomacrogol 1000 solutions containing hydrocortisone before and after irradiation, it is clear from table 3.5.9a that there is a considerable difference in the CMC's due to the presence of the corticosteroid as well as due to irradiation. Therefore the presence of radiolytic products of either the hydrocortisone or of the surfactant monomers may affect the CMC and consequently affect the sensitivity of the corticosteroid to radiation.

With regard to the effect of the three surfactants on the sensitivity of hydrocortisone phosphate to gamma-radiation it is evident from figure 3.5.2 that all three surfactants protect hydrocortisone phosphate and the degree of

protection is again in the order of NaLS > CTAB > Cetomacrogol 1000. Therefore again one may conclude that the surfactants generally compete preferentially with the radiolytic products of water and therefore protect the hydrocortisone phosphate molecules. In the case of NaLS this protection reaches a steady state at an abrupt change in its protective effect at a concentration of 3.9×10^{-3}M as shown in figure 3.5.2 and table 3.5.8 after which NaLS has no further effect. Thus NaLS probably protects hydrocortisone phosphate in a similar manner to its protective effect on hydrocortisone. However, it is evident from the same figure 3.5.2 that CTAB does not behave in a similar manner to hydrocortisone as there appears to be no abrupt change at the determined CMC (1.14×10^{-5}M) of CTAB with hydrocortisone phosphate and radiolytic products as shown in table 3.5.7. This can be explained by the fact that the positive charge on the CTAB micelles will attract the negatively charged hydrocortisone phosphate ions which are then no longer available for ready attack by the radiolytic products of water as the corticosteroid ions are solubilised within the micelles.

The effect of the non-ionic surfactant cetomacrogol 1000 on hydrocortisone phosphate in figure 3.5.2 is the same as that for hydrocortisone as in figure 3.5.1 except that the degree of protection is slightly greater. No break in the curve has been observed in either case, so one can conclude that a similar mechanism of protection for both corticosteroids

is afforded to a small extent by the non-ionic surfactant.

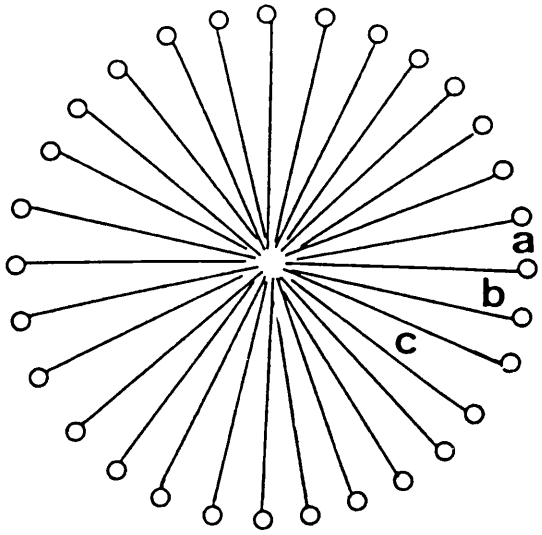
The apparent effect, so far observed, of the three types of surfactants on the sensitivity of hydrocortisone and hydrocortisone phosphate to radiation is actually the combined result of the interaction of each of these surfactants with the three major radiolytic products of water namely the hydroxyl radical, the hydrogen atom and the hydrated electron. Therefore, these observed protective effects are really a net effect of each surfactant's aggregated effects on the individual reaction of each radiolytic product of water with the corticosteroids. Therefore it was necessary to investigate the individual effect of $\dot{O}H$, $\dot{H}$ and $\bar{e}aq.$ on the two corticosteroids in the presence of the three types of surfactants in order to appreciate these net effects.

Before discussing the results of this investigation it is necessary to digress a little and consider the possible solubilisation sites of hydrocortisone and hydrocortisone phosphate in the micelles of the three surfactants.

Advantage has been taken of changes in the ultraviolet absorption maxima of several solubilisates as a function of solvent polarity in order to determine the mode of solubilisation and the possible position of the solubilisate in the micelle[159]. Resemblance of the absorption spectra of the solubilisate in the micellar phase to that in polar solvents is generally interpreted as implying a polar environment of the substrate in the micelles. Conversely, a similarity between the absorption spectrum in the micellar

solution and that in a non-polar solvent is said to indicate that the substrate is solubilised in a hydrocarbon like environment. Using this technique, it is clear from the obtained results presented in table 3.5.6 and shown in figure 3.5.3 that there is a shift in the λ_{max} of hydrocortisone in solutions of the three surfactants from the λ_{max} of the corticosteroid in the pure aqueous solutions and this shift is at its highest in the case of CTAB solution. Comparing the λ_{max} of hydrocortisone in the solutions of surfactants to that in octanol, it can be observed that the λ_{max} of the corticosteroid in the surfactant solutions shifts to a value near to that in the non-polar solvent octanol, which means that the corticosteroid is probably surrounded by a more non-polar environment than water but not to the extent of the non-polarity of octanol. So it can be suggested in relatively broad terms that hydrocortisone is located in the polyoxyethylene shell of the cetomacrogol micelle, while it locates in the outer layer of the core in the case of NaLS and slightly deeper in the case of CTAB, as shown in figure 4.5. Hydrocortisone phosphate, however being more polar than hydrocortisone could be expected to site itself slightly less deeply into the micelles.

Figures 3.5.4a and b, 3.5.5a and b and 3.5.6a and b show the effect of $\dot{H}$, $\dot{O}H$ and $\bar{e}aq.$ in CTAB, NaLS and cetomacrogol 1000 respectively on the sensitivity of hydrocortisone and hydrocortisone phosphate to gamma-radiation. From figures 3.5.4a and 3.5.4b relating to



a - Polyoxyethylene Shell of Cetomacrogol 1000
b - Deep Penetration in the Palisade Layer (NaLS)
c - Short Penetration in the Core (in CTAB)

Fig. 4.5 Possible Location of Hydrocortisone Within the Micelle

CTAB, it can be observed in the initial part of the curves, which is assumed to be below the CMC of CTAB, that the apparent G^- values of hydrocortisone and hydrocortisone phosphate degradation decrease as the surfactant concentration increases. This protection can be considered to be mainly due to a competition of the surfactant monomers for these three radiolytic products of water. Table 1.3 shows that the reaction rate constant of the $\dot{O}H$ with the CTAB molecules, at surfactant concentrations lower than CMC, is $1.04 \times 10^{10} l\ mol^{-1}\ Sec^{-1}$ while the reaction rate constant with the $\dot{H}$ at the same surfactant concentration is $1.6 \times 10^{8} l\ mol^{-1}\ Sec^{-1}$ [13,110,111]. This would support the competitive mechanism for the protection of the corticosteroids because, as shown in figure 3.5.4a and

3.5.4b, it is clear that both hydrocortisone and hydrocortisone phosphate are more protected from $\dot{O}H$ than from $\dot{H}$. At the higher concentration of CTAB, i.e. above the CMC, hydrocortisone phosphate, which is available as negatively charged anions, can be considered to be solubilised in the micelles near the positively charged polar head groups because of the forces of attraction. This will result in decreasing the mutual repulsion between the polar head groups of the CTAB micelles and will in turn lead to an increase in the aggregation number[79], resulting in more solubilisation of the corticosteroid and consequently more protection against $\dot{O}H$ and $\dot{H}$. This improved stabilisation of hydrocortisone phosphate against $\dot{O}H$ and $\dot{H}$ can be clearly observed in figure 3.5.4b. In the case of the $\dot{O}H$, the protection is afforded by increased concentration below the determined CMC and then extends beyond the CMC without any apparent break in the reaction rate between $\dot{O}H$ and the corticosteroid. This result is surprising as a number of workers[117-120] have reported that the reaction rates of most surfactants with $\dot{O}H$ below the CMC are higher than those above the CMC. Therefore one might have expected a levelling off in the effect of CTAB on $\dot{O}H$ reacting with hydrocortisone phosphate, whereas it has apparently continued at the same increasing rate. However, because of the higher micellar aggregation numbers, as already explained more hydrocortisone phosphate is solubilised and therefore continuously protected as shown in figure 3.5.4b. This explanation also accounts for

the abrupt decrease in the G^- value of hydrocortisone phosphate degradation by $\dot{H}$ above the determined CMC.

Hydrocortisone, on the other hand, being less polar than hydrocortisone phosphate penetrates slightly deeper into the CTAB micelle. However, it does not affect the aggregation number in the micelles as much as hydrocortisone phosphate as it does not possess a negative charge. Consequently the amount of hydrocortisone solubilised by the micelles is probably smaller than that of hydrocortisone phosphate, therefore it can be expected that hydrocortisone is less protected from degradation by the $\dot{H}$ and $\dot{O}H$ than hydrocortisone phosphate. This can be observed in figures 3.5.4a and 3.5.4b where the effect of CTAB micelles on reducing the G^- value of hydrocortisone degradation by $\dot{H}$ is smaller than their effect on reducing the G^- value of hydrocortisone phosphate degradation. Examining the effect of CTAB micelles on the sensitivity of hydrocortisone to $\dot{O}H$, it is clear from figure 3.5.4a that hydrocortisone is less protected by the micelles than by the monomers. This observation is compatible with the reaction rate data in table 1.3, where the reaction rate of $\dot{O}H$ with CTAB monomers is about 5 times as much as its reaction rate with the micelles[13,110,111].

Comparing the abrupt changes in the G^- values of degradation of hydrocortisone and hydrocortisone phosphate by the $\dot{O}H$ and $\dot{H}$ in figures 3.5.4a and b to the measured CMC presented in tables 3.5.7a and b, it can be observed that the change in G^- value of the degradation of hydrocortisone

by $\dot{OH}$ approximately coincides with the determined CMC, while for hydrocortisone phosphate degradation, no abrupt change is observed due to the association of hydrocortisone phosphate to the growing micelles resulting in continuous protection of the corticosteroid as the concentration of the surfactant increases. In the case of $\dot{H}$, the abrupt changes in the G^- value of both hydrocortisone and hydrocortisone phosphate degradation take place at surfactant concentration considerably higher than the determined CMC's. This difference may be due to the growth of micelles by the penetration of surfactant radicals resulting from the interaction of the surfactant molecules with the hydrogen atom.

Studying the effect of NaLS on the sensitivity of hydrocortisone and hydrocortisone phosphate to the individual radiolytic products of water, figures 3.5.5a and 3.5.5b show that at surfactant concentrations below the CMC, both corticosteroids are protected from $\dot{OH}$ and $\dot{H}$ through direct competition of the surfactant monomers with the corticosteroids for $\dot{OH}$ and $\dot{H}$, where the G^- value of the corticosteroids degradation decreases as the concentration of the surfactant increases. As the reactivity of the NaLS monomer to $\dot{OH}$ is higher than its reactivity to $\dot{H}$, it is clear from these figures that both corticosteroids are more protected against $\dot{OH}$ than $\dot{H}$. Above the CMC, hydrocortisone phosphate can be expected to be solubilised near the polar head groups of the micelles because of its high polarity. This location of hydrocortisone phosphate

results in its protection from the attack by $\dot{H}$ as shown in figure 3.5.5b. However, hydrocortisone, being less polar than hydrocortisone phosphate it is expected to be located deeper in the micelles in such a way that the $\dot{H}$ cannot gain easy access to attack the drug. Therefore hydrocortisone seems to be more efficiently protected from $\dot{H}$ than hydrocortisone phosphate as shown by comparing figures 3.5.5a and 3.5.5b.

As previously shown in table 1.3, the reaction rate of $\dot{OH}$ with NaLS monomer is about 7 times greater than its reaction with the surfactant micelle. This would suggest that, above the CMC the aggregated surfactant molecules would no longer be able to provide the same degree of protection as the free monomers provide below the CMC. If, on the other hand, hydrocortisone is associated to the micelles and the $\dot{OH}$ does not gain easy access to it then a continuous decrease in the G^- value would be expected above the CMC. Neither of these explanations corresponds to the results shown in figures 3.5.5a and 3.5.5b. One must take into consideration that above the CMC, the monomer will exist in a dynamic equilibrium with those surfactant molecules associated in the micelle. That is the number of surfactant monomers in the bulk solution above the CMC will remain the same and are therefore available to react with the hydroxyl radical in place of hydrocortisone and hydrocortisone phosphate. As the G^- value of the corticosteroids degradation in the presence of the NaLS above its CMC remains constant, this

means that the same number of corticosteroid molecules are destroyed. This would suggest that not only the corticosteroids in the bulk phase, but also those associated with the micelles are possibly destroyed.

Comparing the abrupt changes in the G^- value of both corticosteroids degradation due to $\dot{H}$ and $\dot{O}H$ shown in figures 3.5.5a and 3.5.5b to the measured CMC's presented in tables 3.5.8a and 3.5.8b, it can be observed that these sudden changes in the G^- value coincide with the determined CMC's of NaLS. This would support the discussed role of micelles in the protection of hydrocortisone and hydrocortisone phosphate from $\dot{H}$ and $\dot{O}H$.

Al-Saden *et al*[123] have found that gamma-irradiation of non-ionic surfactant solutions can lead to polyoxyethylene chain scission. This in turn leads to the formation of mixed micelles between the surfactant and the more hydrophobic degraded species. On the other hand, these degraded species may result in the formation of a large number of organic radicals.

Studying the effect of $\dot{H}$ and $\dot{O}H$ on the sensitivity of hydrocortisone phosphate in the presence of the non-ionic surfactant cetomacrogol 1000, it can be seen from figure 3.5.6b that as the concentration of cetomacrogol increases the G^- value of the corticosteroid degradation due to $\dot{H}$ or $\dot{O}H$ increases, until a certain extent after which an abrupt change in the G^- value takes place. This destructive effect of the surfactant monomers could be attributed to the organic radiolytic products of

cetomacrogol 1000 which may attack the corticosteroid molecules in the solution. If this was simply the case, the G^- value of hydrocortisone phosphate degradation due to $\dot{O}H$ should be higher than that due to $\dot{H}$ because the reaction rate of $\dot{O}H$ with the surfactant monomers is higher than the reaction rate with $\dot{H}$ as shown in table 1.3 which would suggest that more organic radicals could be expected to be produced from the reaction of $\dot{O}H$ with surfactant monomers. But as shown in figure 3.5.6b the G^- value of hydrocortisone phosphate degradation due to $\dot{H}$ is higher than that caused by $\dot{O}H$. Two possible reasons could account for the higher G^- value of hydrocortisone phosphate degradation in the presence of $\dot{H}$. The first one is that the radiolytic products resulting from the reaction of $\dot{H}$ with the cetomacrogol 1000 monomers are different from those produced by the reaction with $\dot{O}H$. In other words, hydrocortisone phosphate is more sensitive to the radiolytic products resulting from the reaction of $\dot{H}$ with the surfactant monomers than those produced by reaction with $\dot{O}H$. Another reason is the acidic medium required for the production of $\dot{H}$ may be another factor for the destruction of the surfactant monomers, resulting in a higher yield of the attacking species which cause the degradation of the corticosteroid. At higher concentrations of the surfactant, an abrupt change in the G^- value of hydrocortisone phosphate degradation due to $\dot{O}H$ and $\dot{H}$ can be observed. This change could be due to micellisation of the surfactant molecules with the consequent association of

the corticosteroid to the micelles and its removal from the bulk solution. This is manifested, as shown in figure 3.5.6b, as a sharp decrease in the G^- value of the corticosteroid degradation due to $\dot{H}$ and $\dot{O}H$. It can also be seen from figure 3.5.6b that hydrocortisone phosphate is more efficiently protected from $\dot{O}H$ than $\dot{H}$ and this could be attributed to the same reasons mentioned before, in addition to the higher reaction rate of $\dot{O}H$ with the surfactant micelle than $\dot{H}$ as shown in table 1.3 which results in more protection of the corticosteroid by the direct competition between the micelle and the $\dot{O}H$ for the corticosteroid. Comparing the abrupt changes in the G^- values shown in figure 3.5.6b, to the determined CMC's presented in table 3.5.9b, it can be seen that they coincide in the case of $\dot{H}$ while the change in the G^- value comes at a higher concentration than the determined CMC in the case of $\dot{O}H$. This could be due to the higher reactivity of $\dot{O}H$ than $\dot{H}$ with the surfactant micelles, which means that at the CMC, $\dot{O}H$ still has a high ability to produce the destructive species by reaction with the micelles. As the surfactant concentration increases the number of aggregates in the micelle increases by the inclusion of the surfactant degradation products into the micelle and as a result of this micelle's growth, more protection can be offered to the corticosteroid.

In the case of hydrocortisone, figure 3.5.6a, shows that the corticosteroid is continuously protected against both $\dot{H}$ and $\dot{O}H$ as the surfactant concentration increases. The only difference between hydrocortisone and hydrocortisone

phosphate is the presence of a phosphate group in the side chain which, in some way, appears to increase the sensitivity of the corticosteroid to the radiolytic products of surfactant molecules. Therefore, in the case of hydrocortisone where there is no phosphate group present in the side chain, it appears to be more stable to these attacking species. So the mechanism of protection of hydrocortisone, at low concentration of surfactant, could be due to simple direct competition between the surfactant monomers and the corticosteroid for $\dot{H}$ or $\dot{O}H$. At higher concentration of surfactant, above CMC, hydrocortisone could be expected to associate to the micelles resulting in more protection against the attacking species as shown in figure 3.5.6a.

Figures 3.5.4a and b, 3.5.5a and b and 3.5.6a and b, show the effect of CTAB, NaLS and cetomacrogol 1000 respectively on the sensitivity of hydrocortisone and hydrocortisone phosphate to the hydrated electron. It is clear from these figures that there is no significant change in the G^- values of the corticosteroids' degradation obtained for the solutions containing the three types of surfactants, over the concentration range studied, from the G^- value of the corticosteroids degradation in the absence of the surfactants presented in table 3.3.5. This would suggest that the hydrated electron does not readily react with the surfactants, and the low G^- values obtained reflect its poor reactivity with both corticosteroids compared to that obtained for the hydroxyl radical or hydrogen atom.

From the same figures, no abrupt change in the effect of the surfactants is observed as was observed for the hydroxyl radical and hydrogen atom. This would suggest that the G^- value of the corticosteroids degradation is constant below and above the CMC's, indicating the same degree of decomposition of the corticosteroids before and after micellisation.

The determined CMC's of CTAB and NaLS in the presence of the hydrated electron are presented in tables 3.5.7a and b and 3.5.8a and b respectively, which show that micellisation takes place at slightly lower concentrations of surfactants than in the normal aqueous solutions. Backlund *et al*[102] found that water soluble alcohols such as methanol and ethanol are predominantly dissolved in the water phase and the CMC is lowered because of the reduction of the free energy of the micelle due to the diluted surface charge density on the micelle. This would explain the low CMC's of CTAB and NaLS determined in the presence of the hydrated electron where methanol has been added to remove the $\dot{O}H$.

In the case of the non-ionic surfactant cetomacrogol 1000, tables 3.5.9a and b show that the determined CMC's are slightly higher than that in the normal aqueous solutions. This is also due to the methanol content as Green[103] has reported that methanol and ethanol cause an increase in the CMC of the non-ionic surfactant systems and this effect is attributed to the weakening of the hydrophobic bonding between the surfactant molecules.

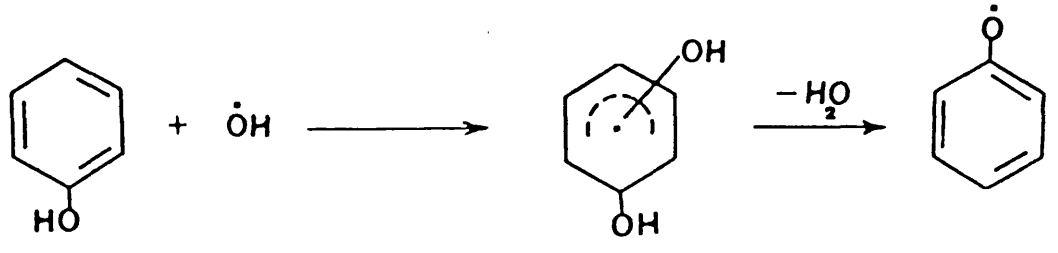
Bansal and colleagues[111] have shown that the degradation of benzene by the hydrated electron is increased

8-fold in the presence of CTAB above its CMC. This result was explained in terms of the electrostatic interaction between the π-electron system of benzene and the net positive charge on the CTAB surface which renders benzene more susceptible to nucleophilic attack of the hydrated electron which is also attracted to the positively charged micelle. For the same reason, Fendler and Patterson[13] have reported that a 3-fold decrease in the rate constant for electron addition to benzene occurred curvilinearly as a function of NaLS concentration because of the electrostatic hindrance of the hydrated electron penetration by the negatively charged NaLS micelles. However, our results do not show any increased or decreased effect due to CTAB or NaLS and this suggests that even if the hydrated electron had access to the corticosteroid molecules associated with the micelle, no significant change in the G^{-} value of the corticosteroids degradation could be observed.

It is clear from all the above results that the three types of surfactants have a maximum protective effect for hydrocortisone and hydrocortisone phosphate above their respective CMC's against the $\dot{O}H$ and $\dot{H}$. These apparent protective effects of the surfactants on the sensitivity of the two corticosteroids to the individual radiolytic products of water explain the observed net combined effects when all three species are present as discussed earlier.

Table 3.6.3 shows the sensitivity of hydrocortisone in the B.P. formulated creams with and without chlorocresol

to gamma-radiation. It is evident from the presented results that hydrocortisone is stable to radiation up to a dose of 27 K.Gray, where only 3-5% of the corticosteroid is destroyed. There are several factors which may be responsible for this apparent stability of the corticosteroid in the formulated creams. Firstly, the creams contain less water than in an aqueous solution and hence may contain less radiolytic products of water to cause degradation. Secondly, hydrocortisone will partition between the oily phase and the aqueous phase of the cream base to an extent governed by its partition coefficient. It could be expected therefore that the portion of the drug present in the non-aqueous phase is larger than that in the aqueous phase due to its non-polar characteristics and low solubility in water, with consquent low sensitivity to radiation. Thirdly, the creams contain 1.8×10^{-1}M of cetomacrogol 1000, a concentration which is well above its CMC (7.7×10^{-5}M) and therefore retards to its maximum the degradation of hydrocortisone in the aqueous phase by radiolytic products of water. In addition to these three reasons, there is also a possible protective effect by the chlorocresol which is present in the formula. It has been reported[46,47,48] that the hydroxyl radical has a considerable reactivity to phenol and its substitutes and phenoxyl type radicals are produced by the addition to the aromatic ring as follows:



It is more than likely that chlorocresol will react with the hydroxyl radical in the same way as phenol resulting in more protection to the corticosteroid. All these factors are probably responsible for the acceptable stability of hydrocortisone in the formulated cream shown in table 3.6.3.

Table 3.6.4 shows the sensitivity of hydrocortisone in different ointment formulations to gamma-radiation and it can be seen that the corticosteroid is stable to radiation up to a dose of 27.15 K.Gray, where only about 3.5-6.5% of the corticosteroid is destroyed, a percentage which is acceptable by the British Pharmacopoeia. The stability of hydrocortisone in the ointment bases could be attributed to several possible reasons. Firstly, the ointment base in the B.P. consists of a mixture of liquid paraffin and soft paraffin which is likely to be highly resistant to radiation because of their long hydrocarbon chain content. Secondly, the percentage of propylene glycol (0.75% w/w) in the Nordic Pharmacopoeia formula is considered to be relatively low for the production of the destructive organic radicals as shown in the earlier study of the effect of propylene glycol on the sensitivity of the hydrocortisone to radiation. This view is supported by the results obtained which show the effect of gamma-radiation on the sensitivity of hydrocortisone in these ointment preparations which include different concentrations of propylene glycol as shown in figure 3.6.1. It is apparent from these results that

the greater the amount of propylene glycol, the greater the degree of degradation of hydrocortisone that occurs. Hayes[6] has reported similar results on studying the effect of propylene glycol on the sensitivity of beclomethasone dipropionate in ointment to gamma-radiation. Thirdly, the presence of cetyl alcohol in the Nordic Pharmacopoeia formula, as shown in table 3.6.4 appears to afford some protection to hydrocortisone against radiation as the corticosteroid has a higher sensitivity to radiation when the cetyl alcohol is excluded from the formula. This apparent protection may be a result of a higher viscous consistency conferred on the formula by the cetyl alcohol's presence, which slows down the diffusion of the attacking species and hence their possible collision with the corticosteroid molecules. Another possibility is that cetyl alcohol molecules are preferentially attacked compared to the hydrocortisone molecules and therefore offer an indirect protection.

All these results, obtained for the ionising radiation of formulated ointments and creams of hydrocortisone would suggest that such products can be subjected to sterilising doses of radiation of 25 K.Gray without a significant loss of the corticosteroid and would therefore probably comply with the specifications of the British Pharmacopoeia regarding an acceptable reduction in the corticosteroid content.

Figures 3.7.1a,b show HPLC traces for the irradiation of hydrocortisone in propylene glycol at 20 K.Gray and in

water at 3 K.Gray. It is evident from both these traces that 4 peaks were observed after these respective periods of irradiation including one peak for the parent drug, hydrocortisone. This would indicate that the 3 other peaks were probable degradation products two of which are small and according to column 2 and 3 of table 3.7.1 have, in both systems, higher capacity factors of 7.08 and 7.66 compared to the capacity factor of 4.41 for hydrocortisone. These values indicate that these two degradation products must have a lower polarity than hydrocortisone. This lower polarity could mean that these degradation products have different functional groups to the parent corticosteroid or they are larger in molecular weight. The third observed peak in the propylene glycol and in the aqueous systems do not have the same capacity factor as indicated by figure 3.7.1 and table 3.7.1 which show that this third peak in the propylene glycol is well separated from hydrocortisone and has a capacity factor of 1.66 whereas the third peak in the aqueous solution is very close to the hydrocortisone peak and has a capacity factor of 4.00. This would suggest that each system on irradiation has produced at least one detectable degradation product of higher polarity and therefore could be smaller than hydrocortisone in molecular weight, but that these degradation products are different for each irradiated solvent system indicative of possibly different or partially similar degradation pathways as the other detected degradation products were the same for both systems.

Figure 3.7.2 and column 5 of table 3.7.1, showing the detected peaks and capacity factors respectively for irradiated hydrocortisone acetate, indicate two degradation products, one of which is close to the peak of hydrocortisone acetate, and has also a capacity factor of 7.08 identical to one of the degradation products already observed for hydrocortisone in both the irradiated aqueous and propylene glycol solutions. As this peak is relatively close to that of hydrocortisone acetate, but of higher polarity, it must have a molecular weight slightly less than that of the ester. However, it is obvious from the data that this degradation product is not hydrocortisone nor is the other observed degradation product which has a capacity factor of 3.58 compared to the capacity factor of 4.41 observed for hydrocortisone. These two detected degradation products from irradiated hydrocortisone acetate in propylene glycol would possibly suggest that the degradation pathway is partially similar to that of irradiated hydrocortisone in propylene glycol as at least one of the degradation products are identical for both corticosteroids.

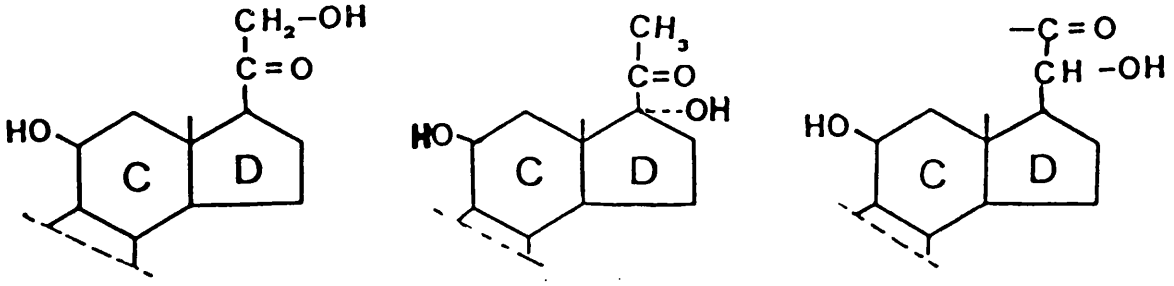
Examining figures 3.7.3a,b and column 3 and 4 of table 3.7.1, it is evident that hydrocortisone phosphate irradiated in either water or propylene glycol produced 3 detected degradation products two of which, although not well resolved, are of similar capacity factors as the two degradation products observed for irradiated hydrocortisone solution and to one degradation product of the

irradiated hydrocortisone acetate. All these results together would suggest that probably these corticosteroids undergo similar pathways initially, but thereafter further degradation products by diverging pathways occur and it is these secondary degradation products that are being detected.

However, it must be cautiously noted from the presented figures that not all the peaks are well resolved and could be masking or coinciding with other degradation products. It must also be remembered that the peaks observed are only detected because they absorb in the u.v. at 248 nm and therefore other degradation products which do not absorb at this wavelength will have been missed.

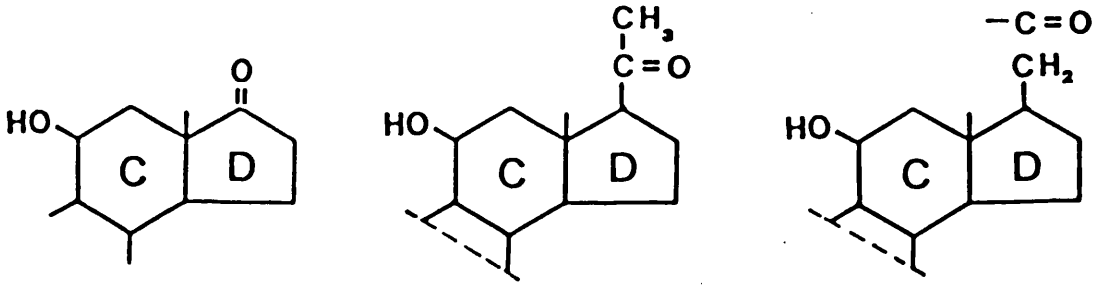
For the purpose of investigating further the nature of the degradation products, TLC separation followed by detection under u.v. light at 254 nm. and with tetrazolium blue reagent was carried out for the three corticosteroids irradiated in propylene glycol for 20 K.Gray. Table 3.7.2 and figure 3.7.3, show the possible degradation products and their corresponding R_f values obtained from chromatograms viewed under the u.v. light and sprayed with alkaline tetrazolium blue reagent. One spot(4) having R_f of 0.65 has been detected and found to be common in all three solutions of corticosteroids and it is possible that it is the same product which appeared as one peak in the HPLC trace of hydrocortisone acetate and appeared as a split peak in the case of the other two corticosteroids. This spot absorbs

u.v. at 254 nm. which is close to 248 nm. of HPLC chromatogram and also gave a positive result with tetrazolium blue reagent indicating that both the chromophoric group in ring A and the $-COCH_2OH$ side chain are still intact or at least there is still a carbonyl group with an adjacent hydroxyl group connected to ring D giving the following possible compounds:



A second degradation product, spot (2), having an R_f value of 0.37-0.39 was also found to be common to all three corticosteroids, but it is obviously more polar in nature than hydrocortisone and less polar than hydrocortisone phosphate. Having positively responded to tetrazolium blue reagent and absorbed in u.v. at 254 nm., it can be concluded that this compound has the chromophoric group in ring A and a carbonyl group adjacent to a hydroxyl group in the side chain. The absence of this compound in the HPLC traces could be due to its being masked by either the parent drug or other degradation products, or it is in such small amounts that it could not be detected in the HPLC trace especially if the wavelength

of its maximum absorbance is not 248nm. The spots (1) detected on the base line are not likely to be traces of the undegraded drugs retained at the base line because of two reasons. Firstly, the authentic samples of both hydrocortisone and hydrocortisone acetate as controls completely moved away and have been detected by u.v. and tetrazolium blue without leaving any traces. Secondly, the authentic sample of hydrocortisone phosphate as a control gave an expected negative result to tetrazolium blue while the spotted irradiated sample gave a positive result indicating that the spot on the base line is not only hydrocortisone phosphate. Therefore, these spots on the base line must be very polar compounds and have chromophoric groups and the $-COCH_2OH$ side chain. Two other degradation products (spots 5,6) have been detected by u.v., but they did not respond to spraying with tetrazolium blue reagent, indicating that the side chain has been affected by radiation resulting in the following possible degradation products.

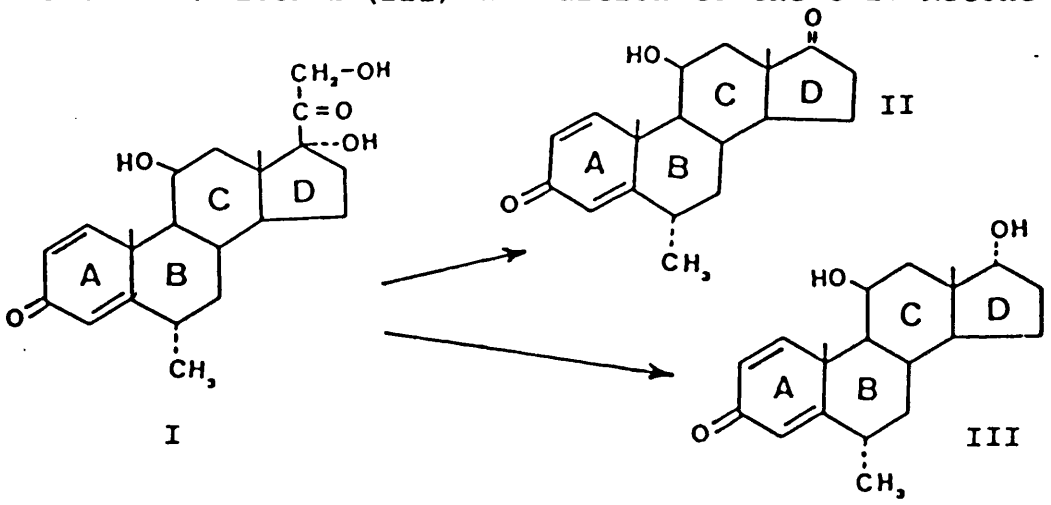


Because of the high polarity of hydrocortisone phosphate and its subsequent strong bonding on the base line of the TLC plate and the need to separate the highly polar

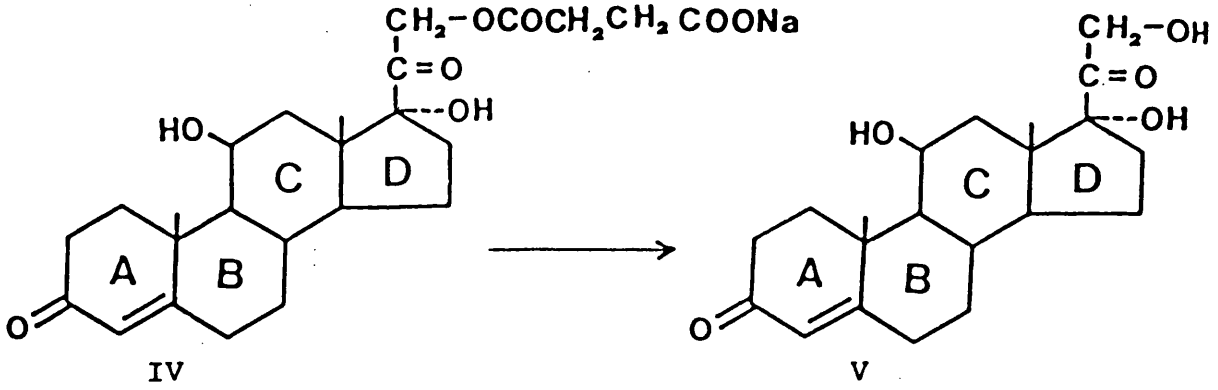
degradation products, strongly bound to the straight phase silica gel, reversed phase TLC separation for the irradiated corticosteroids was carried out. The observed degradation products and their corresponding R_f values obtained from the chromatograms and viewed under u.v. light or sprayed with tetrazolium blue reagent are presented in table 3.7.3 and are shown in figure 3.7.4. One spot for each drug has been detected on the base line by u.v. at 366 nm. indicating that they represent non-polar and large molecular size compounds having strong chromophoric groups which could possibly be dimers of the parent corticosteroids. Two other identical spots having very low R_f values have been detected in the case of hydrocortisone and hydrocortisone acetate indicating non-polar compounds having the $CO-CH_2OH$ grouping. The two identical spots having R_f value of 0.105 obtained in the case of hydrocortisone and hydrocortisone phosphate may correspond to the split peak obtained in the HPLC traces. The two identical spots having R_f values of 0.165 obtained in the case of hydrocortisone phosphate and hydrocortisone acetate are very close to hydrocortisone (R_f = 0.166), indicating that hydrocortisone may be one of the degradation products of the corticosteroid esters, but in such a minor amount that it could not be detected in the HPLC traces.

Hydrocortisone acetate, isoflupredone acetate, methyl prednisolone acetate and prednisolone were subjected to gamma-radiation up to the sterilisation dose by many workers[2,3] who showed that two major types of radiolytic

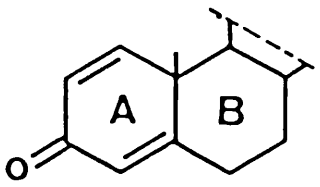
degradation schemes were found to occur. The first scheme is through the loss of the corticosteroid side chain on the D-ring to produce the C-17 Ketone, while the second scheme is through the conversion of the C-11 alcohol to the C-11 Ketone. However, minor degradation products, derived from other changes affecting the side chain, were also identified by the same workers. For example, the loss of the C-17 side chain in methyl prednisolone (I) was found to result in the formation of the C-17 α alcohol (III) in addition to the C-17 Ketone (II).



Also, hydrocortisone (V) was identified as an additional degradation product of hydrocortisone sodium succinate (IV).



The last two spots of R_f values of 0.208 and 0.430 obtained in the case of irradiated hydrocortisone and hydrocortisone phosphate respectively gave positive results with tetrazolium blue reagent and detected by u.v. light at 366 nm., indicating that they have $CO-CH_2OH$ group and have strong chromophoric groups which could be on ring A as the following example:



The tail shown in the separation of the irradiated hydrocortisone sample represents traces of a group of polar degradation products which are not well separated.

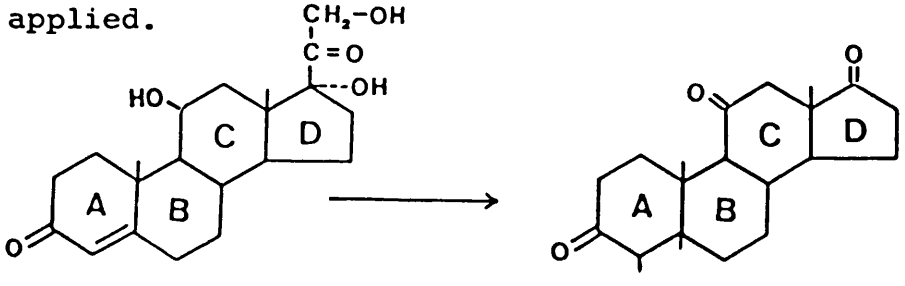
From both straight phase and reverse-phase TLC, it is evident that the irradiation of hydrocortisone in propylene glycol results in a yield of four main degradation products, two of which have the $CO-CH_2OH$ group intact and one of these two products is more polar than the parent corticosteroid. In the case of hydrocortisone phosphate, three main degradation products were detected, two of them are identical to those of hydrocortisone and the third has a higher polarity than the parent drug. On separating the degradation products of hydrocortisone acetate, three main products could be detected, two of them are identical to those of hydrocortisone. Therefore, it may be concluded that the three corticosteroids probably have similar

degradation pathways which may diverge as the irradiation reaction proceeds.

In order to obtain larger amounts of the degradation products separated by TLC and to ascertain if these degradation products correspond to those previously highlighted in the standard HPLC traces, further thick layer separation of large amounts of the three corticosteroids irradiated in propylene glycol was carried out. Figure 3.7.5 shows the separation of the three corticosteroids and their degradation products after irradiation in propylene glycol for 40 K.Gray. Definite zones of separation were observed and the products that were detected at 254 nm. and 366 nm. were scraped off, extracted, concentrated and injected onto the HPLC column. From figure 3.7.5, it can be seen that bands 4 and 5 are nearly identical in the irradiated hydrocortisone and hydrocortisone phosphate solutions while they are not detected in the irradiated hydrocortisone acetate solution. Also, it can be observed that the band number 6 is common to the three corticosteroids and absorbs u.v at 366 nm. indicating that it is a very large non-polar molecule having stronger or more conjugated chromophoric groups than the parent corticosteroid. Injecting each of these extracted bands onto the HPLC column, the traces shown in figures 3.7.6, 3.7.7 and 3.7.8 could be detected. It can be observed that all the peaks expected to appear before hydrocortisone have been masked by the impurities extracted from the stationary phase of the chromatoplates. On the other hand, it is evident that peaks c and d which

represent the bands number 4 and 5 are identical in both hydrocortisone and hydrocortisone phosphate systems and have the same K' as those obtained in the first HPLC traces (6.25 and 7.00). The band number (6) could not be detected in the HPLC trace indicating that this compound may have a very high retention time with respect to the HPLC system used and was not detected within the observed time span. This would support that it may be a dimer of the parent corticosteroid.

These investigations by HPLC and TLC have revealed that there are a large number of possible degradation products, most of them are formed as a result of secondary degradation of the primary products, making the identification of these degradation products rather difficult. Another difficulty is that the amounts of these degradation products are very small compared to the parent corticosteroid which also makes their separation in reasonable amounts for NMR or IR analysis very difficult. However, the large number of degradation products detected give an indication that most of the reaction sites are limited to rings A and D in addition to the side chain of ring D, figure 4.6. On the other hand, the reactions can take place at both A and D rings of the corticosteroids simultaneously[29] and the resulting degradation products would not be detected by the tests applied.



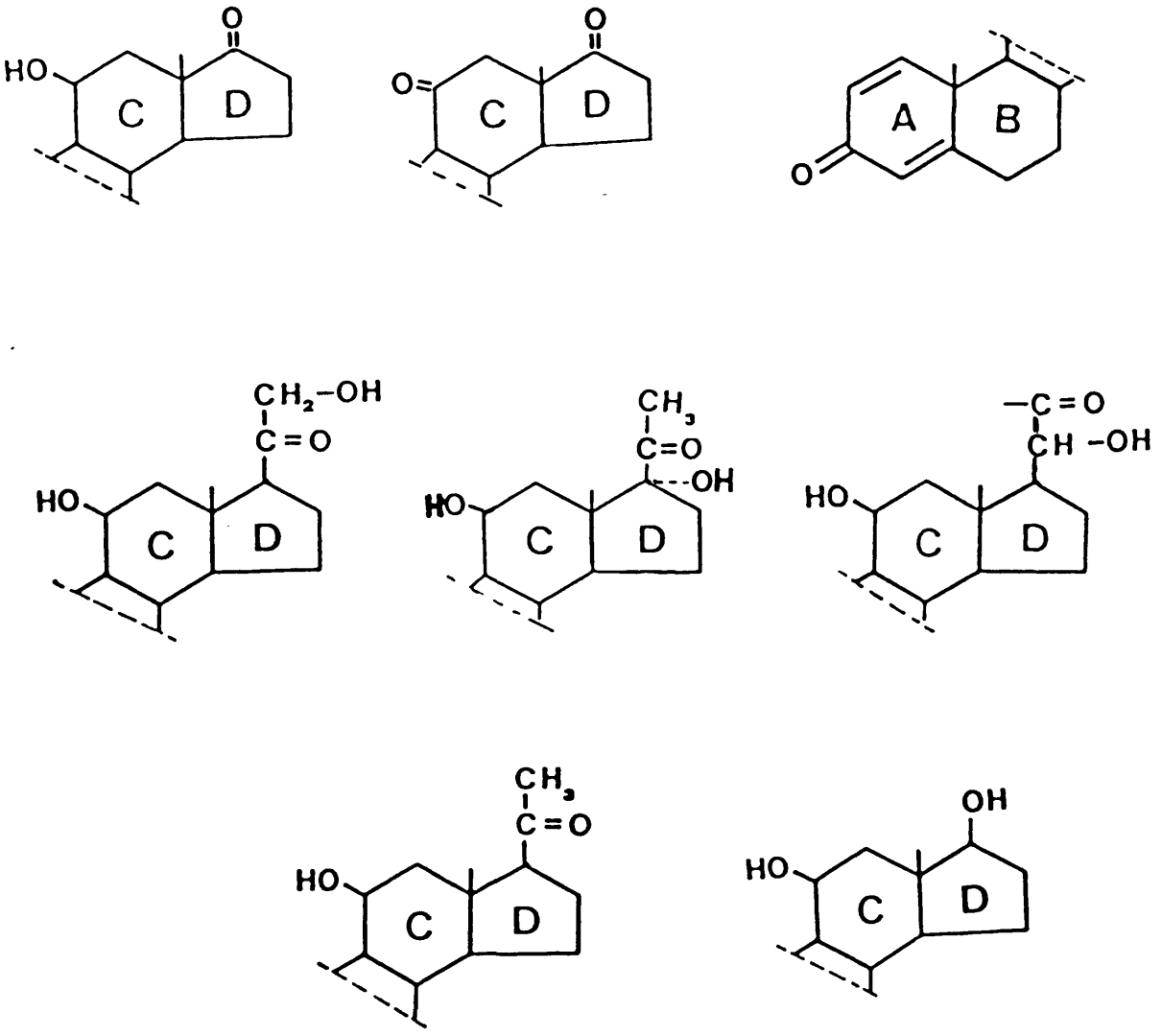


Fig. 4.6 Some of the Possible Degradation Products Expected after Irradiation of Hydrocortisone in Propylene Glycol

It is evident from this study that hydrocortisone formulated and presented as a cream can feasibly be sterilised by gamma-radiation without significant loss of its potency. The presence of, the non-ionic surfactant, cetomacrogol 1000 at the high level of concentration required to act as an emulsifying agent along with cetostearyl alcohol readily protect the drug molecules in the aqueous phase from the attacking radiolytic species of water. The organic components of the cream base such as the paraffin also afford protection to the corticosteroid as they are resistant to radiation effects and the fact that the drug is predominantly dispersed as a suspension in the cream vehicle reduces the probability of indirect action of free radicals. Similarly when the corticosteroid is formulated as a dispersion in a paraffin base to be presented as an ointment, the drug is in a non-aqueous environment which is comparatively stable to gamma-radiation and affords adequate protection to the corticosteroid. However, if certain solvents such as propylene glycol are used as pharmaceutical adjuvants to aid dispersion or percutaneous absorption, the corticosteroid's stability can be adversely affected. This has been shown by Hayes[6] to be the case for Beclomethasone Dipropionate and has been borne out by this study on Hydrocortisone and its acetate ester.

This study, however, has not taken into account the possible toxicity of the degradation products resulting by radiation. This would have to be investigated even

although their apparent concentration are relatively small to ensure adequate quality and safety of such products after sterilisation by gamma-radiation.

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APPENDICES

APPENDIX I

THE DECAY FACTOR FOR COBALT-60

DAYS	FACTOR	DAYS	FACTOR
1	0.9996	60	0.9786
2	0.9993	100	0.9645
3	0.9989	200	0.9340
4	0.9986	300	0.8975
5	0.9982	400	0.8656
10	0.9964	500	0.8349
20	0.9928	600	0.8053
30	0.9892	700	0.7763

APPENDIX II

STATISTICAL ANALYSIS

Least Squares Regression Analysis

When a linear relationship is assumed to exist between two variables it is usual to fit a straight line by a least squares regression analysis. The simplest statistical model for this assumes that the independent variable X is known without error of measurement, and that the corresponding measured values of the dependent variable Y are scattered normally from their true values. Hence, each value Y_i of the dependent is normally distributed with a mean, $\alpha + \beta X_i$.

The method of least squares obtains estimates of a and b in the equation $Y = a + bX$ such that the sum of the squares of the deviations of the observations Y_i from their mean of $\alpha + \beta X_i$ is a minimum.

These values are:

$$a = \frac{\Sigma Y_i - b\Sigma X_i}{n} = \bar{Y} - b\bar{X}$$

$$b = \frac{n\Sigma X_i Y_i - \Sigma X_i \Sigma Y_i}{n\Sigma X_i^2 - (\Sigma X_i)^2}$$

$$= \frac{\sum(X_i - \bar{X})(Y_i - \bar{Y})}{\sum(X_i - \bar{X})^2}$$

where n = number of points on the line.

Variance of the Slope (b)

This is termed S_b^2 and is given by the equation:

$$S_b^2 = \frac{\sigma e^2}{\sum(X_i - \bar{X})^2}$$

where σe^2 is the residual variance of the dependent variable Y and is obtained from:

$$\sigma e^2 = \frac{\Sigma D^2}{n-2}$$

where ΣD^2 is the residual sum of squares. ΣD^2 is obtained from the equation:

$$\Sigma D^2 = \Sigma(Y_i - \bar{Y})^2 - \frac{\left[\sum(X_i-\bar{X})\ (Y_i-\bar{Y})\right]^2}{\Sigma(X_i-\bar{X})^2}$$

$$= \Sigma(Y_i - \bar{Y})^2 - b^2\ \Sigma(X_i-\bar{X})^2$$

The denominator (n-2) shows that two degrees of freedom have been lost because both the slope and intercept were estimated from the data. The standard deviation of the slope is given by the square root of variance.

Variance of the Intercept (a)

This is termed:

$$S_a^2 = \frac{\Sigma X_i^2\ \sigma e^2}{n\Sigma(X_i-\bar{X})^2}$$

where $\sigma e^2 = \frac{\Sigma D^2}{n-2}$

The standard deviation of the intercept is given by the square root of the variance.

Correlation Coefficient

The correlation coefficient r is defined as:

$$r = \frac{\Sigma(X_i - \bar{X})\ (Y_i - \bar{Y})}{\sqrt{\Sigma(X_i - \bar{X})^2\ \Sigma(Y_i - \bar{Y})^2}}$$

To represent a linear relationship between two variables, X and Y, r must be $\pm$ unity. The calculated value of r is compared with the tabulated value at the 5% probability level for n-2 degrees of freedom, and if found to be greater than the tabulated value, the observations were considered to be linearly related.

The above computations were carried out using a Fortran program on an ICL 4.50 computer.

To Determine the Equality of Two Estimates of a Parameter (t-Test)

The equality of estimates P_1 and P_2 with respective variances S_1^2 and S_2^2 of a parameter P is assessed by means of the following statistic:

$$t = \frac{P_1 - P_2}{\sqrt{S_1^2 + S_2^2}}$$

The value of t is compared with tabulated values with $n_1 + n_2 - 4$ degrees of freedom where n_1 and n_2 are the number of observations used in the estimation of P_1 and P_2 respectively. If the value of t does not exceed the

tabulated value of the 5% probability level, the parameters are assumed to be indistinguishable at that probability level.

Equality of More Than Two Estimates of a Parameter

Bartlett Test)

When more than two estimates of a parameter P are tested for equality the following statistic is used:

$$B = \frac{\sum(P_1 - \bar{P})^2}{\sigma^2}$$

If the estimates of P_1 all come frome the same normal distribution $\frac{(P_1 - \bar{P})^2}{\sigma^2}$ will have a X^2 distribution with n-1 degrees of freedom where n is the number of estimates and σ^2 is given by the expression

$$\sigma^2 = \frac{w_1 S_1^2 + w_2 S_2^2 + \ldots\ldots w_n S_n^2}{w_1 + w_2 + \qquad w_n}$$

where S_1, S_2 etc. are the standard errors associated with the estimates P_1, P_2 etc. and w_1, w_2 etc. are the number of observations used in determining the estimates.

Analysis of Variance (F-test)

The method known as analysis of variance considers the problem of determining whether, among a set of three or more samples, there are means that differ significantly. We let K denote the number of samples.

Sample 1 contains n_1 variates denoted $A_{1\,1}$, $A_{2\,1}$, ... $A_{n_1 1}$.

Sample 2 contains n_2 variates denoted $A_{1\ 2}$, $A_{2\ 2}$, ... $A_{n_2\ 2}$ and so on as seen in table I. In a sense, this method is a generalisation of the test which is used to determine whether the means of two given samples differ significantly.

Table I Arrangement of Data for K Samples with Unequal Sample Sizes, n_1, n_2, ... n_K

	1st Sample	2nd Sample		Kth Sample
	$A_{1\ 1}$	$A_{1\ 2}$	....	$A_{1\ K}$
	$A_{2\ 1}$	$A_{2\ 2}$	....	$A_{2\ K}$
	$A_{3\ 1}$	$A_{3\ 2}$	....	$A_{3\ K}$
	.	.		.
	.	.		.
	.	.		.
	$A_{n_1 1}$	$A_{n_2 2}$		$A_{nK\ K}$
Total	T_1	T_2		T_K
Mean	$\bar{A}_1$	$\bar{A}_2$		$\bar{A}_K$

Grand Total = T

Grand Mean = $\bar{A}$

From the sample values, we determine the total and the mean for each sample and the mean of all means, called the grand mean $\bar{A}$. The total sum of squares, S.S., is the sum of the squared differences of each variate

from the grand mean $\bar{\bar{A}}$. Thus, S.S. is given by

$$S.S. = (A_{1\ 1} - \bar{\bar{A}})^2 + (A_{2\ 1} - \bar{\bar{A}})^2 + \ldots\ldots\ (A_{nK\ K} - \bar{\bar{A}})^2$$

An alternate form, more suitable for calculation, will be given subsequently. Also, we define the between means sum of squares, denoted S.S.T., by the relation

$$S.S.T. = n_1(\bar{A}_1 - \bar{\bar{A}})^2 + n_2(\bar{A}_2 - \bar{\bar{A}})^2 + \ldots\ldots\ n_K(\bar{A}_K - \bar{\bar{A}})^2$$

and the within-samples sum of squares, S.S.E., by the relation

$$S.S.E. = \sum_1^{n_1} (A_{1\ 1} - \bar{A}_1)^2 + \sum_1^{n_2} (A_{1\ 2} - \bar{A}_2)^2 + \ldots \sum_1^{n_K} (A_{1K} - \bar{A}_K)^2$$

It may be shown that the total sum of squares for the set of variates is the sum of the between-means sum of squares and the within samples sum of squares

$$S.S. = S.S.T. + S.S.E.$$

The partitioning of S.S. into S.S.T. and S.S.E. is useful in that these provide two estimates of the population variance σ^2. These estimates are denoted by S_p and S_t where

$$S_p^2 = S.S.E./(n_1 + n_2 + n_3 \ldots + n_K - K)$$

and

$$S_t^2 = S.S.T./(K - 1)$$

The ratio S_t^2/S_p^2 satisfies the F-distribution, where

$$F = S_t^2/S_p^2$$

with degree of freedom $V_1 = K - 1$ and $V_2 = n_1 + n_2 + \ldots n_K - K$

The value of F obtained from the equation is compared with the tabular value at the specified level of significance

(usually 95%). If the computed F exceeds the tabular value, then there is at least one pair of samples whose means differ significantly.